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POLYNUCLEOTIDES, POLYPEPTIDES, CELLS, AND METHODS THEREOF CAPABLE OF  
MODULATING VARIOUS RESPONSES

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I.B.3. Chloroplast Genes And Gene Components

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POLYNUCLEOTIDES, POLYPEPTIDES, CELLS, AND METHODS THEREOF CAPABLE  
OF MODULATING VARIOUS RESPONSES

5 This application claims priority under 35 USC §119(e), §119(a-d) and §120 of the  
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United States	2750-1156P	60/228,279	8/25/00
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This application contains a CDR, the entire contents of which are hereby incorporated by  
reference. The CDR contains the following files:

File Name	Date of Creation	File Size
010809 Protein Domain Table	August 09, 2001	2.62MB
2750-1481P AFLP_Diff Table 1	August 23, 2001	14.7KB
2750-1481P AFLP_Diff Table 2	August 23, 2001	8.73KB
2750-1481P AFLP_Diff Table 3	August 23, 2001	4.09KB
2750-1481P AFLP_Diff Table 4	August 23, 2001	7.85KB
2750-1481P AFLP_Diff Table 5	August 23, 2001	27.1KB
2750-1481P AFLP_Diff Table 6	August 23, 2001	24.3KB
2750-1481P AFLP_Diff Table 7	August 23, 2001	18.7KB
2750-1481P AFLP_Diff Table 8	August 23, 2001	36.3KB
2750-1481P AFLP_Diff Table 9	August 23, 2001	16.9KB
2750-1481P AFLP_Diff Table 10	August 23, 2001	33.2KB
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2750-1481P Nitrogen Reference Table 1-02	August 23, 2001	2.81MB
2750-1481P Nitrogen Reference Table 1-03	August 23, 2001	43.9KB
2750-1481P Nitrogen Reference Table 1-04	August 23, 2001	27.0KB
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2750-1481P GA Reference Table 1-07	August 23, 2001	15.8KB
2750-1481P GA Reference Table 1-08	August 23, 2001	22.7KB
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2750-1481P Sequence Table 2-20	August 23, 2001	871KB
2750-1481P AFLP_Int Table 1	August 23, 2001	6.52MB
2750-1481P AFLP_Int Table 2	August 23, 2001	8.60MB

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FIELD OF THE INVENTION

The present invention relates to over 100,000 isolated polynucleotides that include a complete coding sequence, or a fragment thereof, that is expressed. These polynucleotides come from five plant species. In addition, the present invention relates to the polypeptide or protein corresponding to the coding sequence of these polynucleotides. The present invention also relates to isolated polynucleotides that represent regulatory regions of genes. The present invention also relates to isolated polynucleotides that represent untranslated regions of genes. The present invention further relates to the use of these isolated polynucleotides and polypeptides and proteins.



## BACKGROUND AND SUMMARY OF THE INVENTION

There are more than 300,000 species of plants. They show a wide diversity of forms, ranging from delicate liverworts, adapted for life in a damp habitat, to cacti, capable of surviving in the desert. The plant kingdom includes herbaceous plants, such as corn, whose life cycle is measured in months, to the giant redwood tree, which can live for thousands of years. This diversity reflects the adaptations of plants to survive in a wide range of habitats. This is seen most clearly in the flowering plants (phylum Angiospermophyta), which are the most numerous, with over 250,000 species. They are also the most widespread, being found from the tropics to the arctic.

The process of plant breeding involving man's intervention in natural breeding and selection, is some 20,000 years old. It has produced remarkable advances in adapting existing species to serve new purposes. The world's economics was largely based on the successes of agriculture for most of these 20,000 years.

Plant breeding involves choosing parents, making crosses to allow recombination of gene (alleles) and searching for and selecting improved forms. Success depends on the genes/alleles available, the combinations required and the ability to create and find the correct combinations necessary to give the desired properties to the plant. Molecular genetics technologies are now capable of providing new genes, new alleles and the means of creating and selecting plants with the new, desired characteristics.

When the molecular and genetic basis for different plant characteristics are understood, a wide variety of polynucleotides, both endogenous polynucleotides and created variants, polypeptides, cells, and whole organisms, can be exploited to engineer old and new plant traits in a vast range of organisms including plants. These traits can range from the observable morphological characteristics, through adaptation to specific environments to biochemical composition and to molecules that the plants (organisms) exude. Such engineering can involve tailoring existing traits, such as increasing the production of taxol in yew trees, to combining traits from two different plants into a single organism, such as inserting the drought tolerance of a cactus into a corn plant. Molecular and genetic knowledge also allows the creation of new traits. For example, the production of chemicals and pharmaceuticals that are not native to particular species or the plant kingdom as a whole.

The application reports the inventions Applicants have discovered to build a foundation of scientific understanding of plant genomes to achieve these aims. These inventions include polynucleotide and polypeptide sequences, and data relating to where and when the genes are differentially expressed and phenotypic observations resulting from either aberrant gene activation or disruption. How these data are transformed into a scientific understanding of plant biology and the control of traits from a genetic perspective also is explained by the instant application. Applications of these discoveries to create new prototypes and products in the field of chemical, pharmaceutical, food, feed, and fiber production are described herein as well.

The achievements described in this application were possible because of the results from a cluster of technologies, a genomic engine, depicted below in Schematic 1, that allows information on each gene to be integrated to provide a more comprehensive understanding of gene structure and function and the deployment of genes and gene components to make new products.

a) The discoveries of the instant application

Applicants have isolated and identified over one hundred thousand genes, gene components and their products and thousands of promoters. The genes were isolated and/or characterized from arabidopsis, soybean, maize, wheat and rice. These species were selected because of their economic value and scientific importance and were deliberately chosen to include representatives of the evolutionary divergent dicotyledonous and monocotyledonous groups of the plant kingdom. The number of genes characterized in this application represents a large proportion of all the genes in these plant species.

The techniques used initially to isolate and characterize most of the genes, namely sequencing of full-length cDNAs, were deliberately chosen to provide information on complete coding sequences and on the complete sequences of their protein products.

Gene components and products the Applicants have identified include exons, introns, promoters, coding sequences, antisense sequences, terminators and other regulatory sequences. The exons are characterized by the proteins they encode and arabidopsis promoters are characterized by their position in the genomic DNA relative to where mRNA synthesis begins and in what cells and to what extent they promote mRNA synthesis.

Further exploitation of molecular genetics technologies have helped the Applicants to understand the functions and characteristics of each gene and their role in a plant. Three powerful molecular genetics approaches were used to this end:

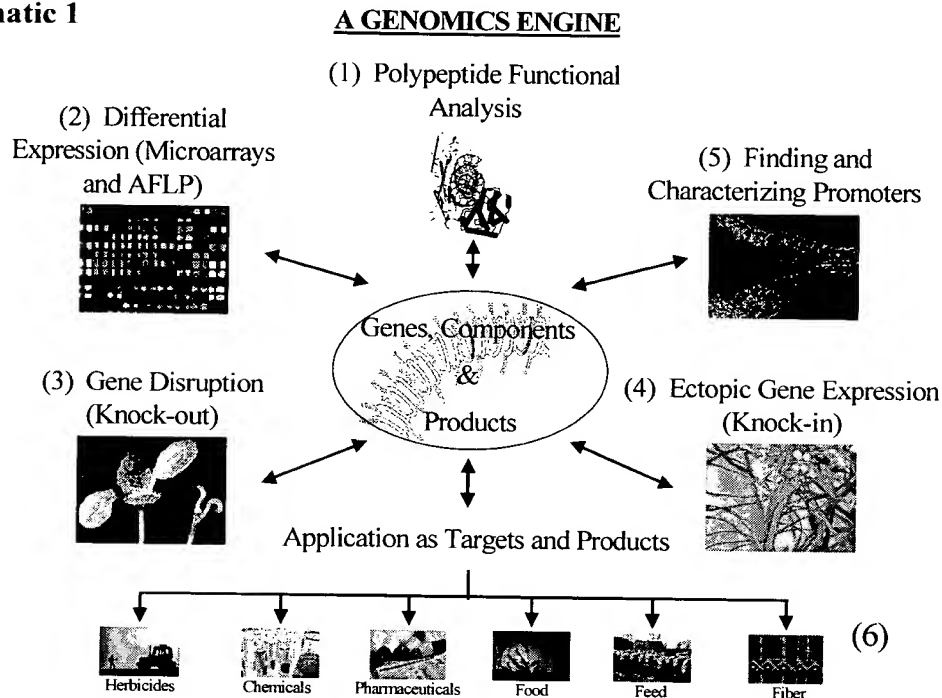
- (a) Analyses of the phenotypic changes when the particular gene sequence is interrupted or activated differentially; (arabidopsis)
- (b) Analyses of in what plant organs, to what extent, and in response to what environmental signals mRNA is synthesized from the gene; (arabidopsis and maize) and
- (c) Analysis of the gene sequence and its relatives. (all species)

These were conducted using the genomics engine depicted in Figure 1 that allows information on each gene to be integrated to provide a more comprehensive understanding of gene structure and function and linkage to potential products.

The species arabidopsis was used extensively in these studies for several reasons: (1) the complete genomic sequence, though poorly annotated in terms of gene recognition, was being produced and published by others and (2) genetic experiments to determine the role of the genes in planta are much quicker to complete.

The phenotypic tables, MA tables, AFLP data table and reference tables and sequence tables indicate the results of these analyses and thus the specific functions and characteristics that are ascribed to the genes and gene components and products.

Schematic 1



*Schematic 1. Gene sequences were determined and are depicted to occupy the center of the figure. Five different sorts of technologies were deployed in the Genomics engine to discover the functions of the genes. (1) Computer-based comparisons of protein structural features. (2) Studies to discover where and when each gene and groups of genes are active. (3) Discovery of the phenotypic consequences of inactivating each gene. (4) Elucidation of the phenotypic consequences of activating a gene in a new way. (5) Discovery of the sequence and activity of promoters of the genes. All this information leads to knowledge of how to use the genes, and gene components to create new products for industrial applications. (6)*

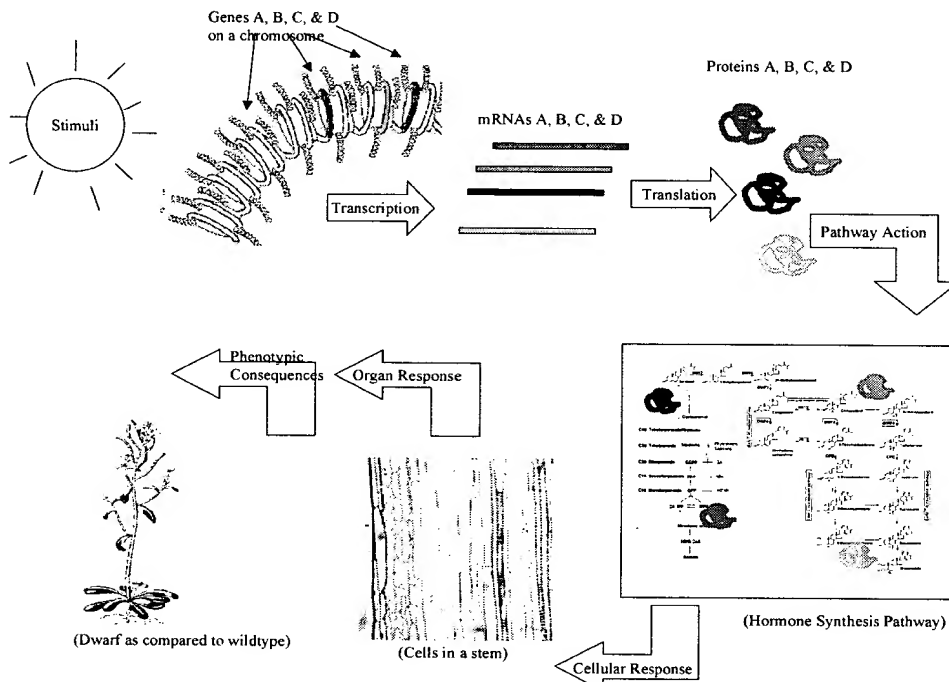
b) Integration of discoveries to provide scientific understanding

From the discoveries made, Applicants have deduced the biochemical activities, pathways, cellular roles, and developmental and physiological processes that can be modulated using these components. These are discussed and summarized in sections based on the gene functions characteristics from the analyses and role in determining phenotypes. These sections illustrate and emphasize that each gene, gene component or product influences biochemical activities, cells or organisms in complex ways, from which there can be many phenotypic consequences.

An illustration of how the discoveries on gene structure, function, expression and phenotypic observation can be integrated together to understand complex phenotypes is provided in Figure 2. This sort of understanding enables conclusions to be made as to how the genes, gene components and product are useful for changing the properties of plants and other organisms.

This example also illustrates how single gene changes in, for example, a metabolic pathway can cause gross phenotypic changes.

**Schematic 2**



*Schematic 2. The figure illustrates how genes A, B, C and D are activated by internal stimuli and then their mRNA transcripts translated into proteins. These proteins are enzymes in three different but linked pathways. All three pathways are activated by the same stimuli. One of them, depicted by the green and light blue proteins determines the levels of a hormone in the shoot meristems causes cells to expand. This cell expansion leads to a longer stem and a taller plant. Genes A & C are therefore useful for controlling plant height and stem strength. The other two*

Furthermore, the development and properties of one part of plant can be interconnected with other parts. The dependence of shoot and leaf development on root cells is a classic example. Here, shoot growth and development require nutrients supplied from roots, so the protein complement of root cells can affect plant development, including flowers and seed production. Similarly, root development is dependent on the products of photosynthesis from

leaves. Therefore, proteins in leaves can influence root developmental physiology and biochemistry.

Thus, the Utility and Application sections describe both the functions and characteristics of the genes, gene components and products and also the multiplicity of biochemical activities, cellular functions, and the developmental and physiological processes influenced by them. The sections also describe examples of commercial products that can be realized from the inventions.

Analyses to Reveal Function and *in vivo* roles of single genes in one plant species.

The genomics engine has focused on individual genes to reveal the multiple functions or characteristics that are associated to each gene, gene components and products of the instant invention in the living plant. For example, the biochemical activity of a protein is deduced based on its similarity to a protein of known function. In this case, the protein may be ascribed with, for example, an oxidase activity. Where and when this same protein is active can be uncovered from differential expression experiments, which show that the mRNA encoding the protein is differentially expressed in response to drought and in seeds but not roots. The gene disruption experiments reveal that absence of the same protein causes embryo lethality.

Thus, this protein is characterized as a seed protein and drought-responsive oxidase that is critical for embryo viability.

Analyses to Reveal Function and roles of single genes in different species.

The genomics engine has also been used to extrapolate knowledge from one species to many plant species. For example, proteins from different species, capable of performing identical or similar functions, preserve many features of amino acid sequence and structure during evolution. Complete protein sequences have been compared and contrasted within and between species to determine the functionally vital domains and signatures characteristic of each of the proteins that is the subject of this application. Thus, functions and characteristics of arabidopsis proteins have been extrapolated to proteins containing similar domains and signatures of corn, soybean, rice and wheat and by implication to all other (plant) species.

Schematic 3 provides an example. Two proteins with related structures, one from corn, a monocot, and one from arabidopsis, a dicot, have been concluded to be orthologs. The known

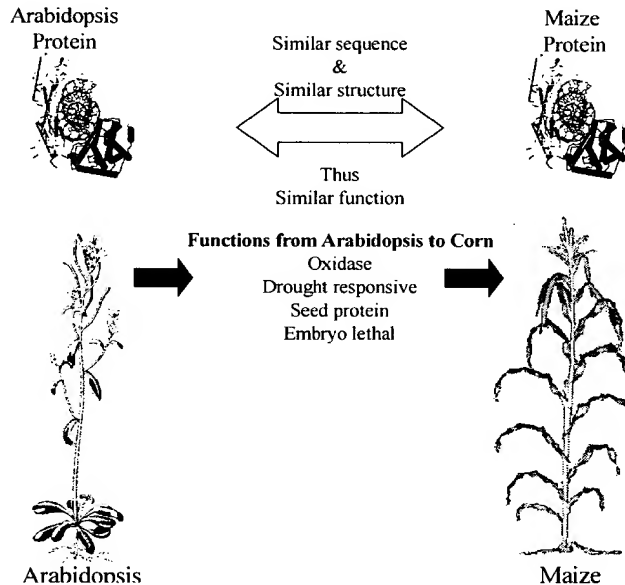
characteristics of the arabidopsis protein (seed protein, drought responsive oxidase) can then be

attributed to the  
protein.

**Integration of Data Across Species to Link Gene Products and Phenotypes**

corn

**Schematic 3.**



Analyses over multiple experiments to reveal gene networks and links across species

The genomics engine can identify networks or pathways of genes concerned with the same process and hence linked to the same phenotype(s). Genes specifying functions of the same pathway or developmental environmental responses are frequently co-regulated i.e. they are regulated by mechanisms that result in coincident increases or decreases for all gene members in the group. The Applicants have divided the genes of arabidopsis and maize into such co-regulated groups on the basis of their expression patterns and the function of each group has been deduced. This process has provided considerable insight into the function and role of thousands of the plant genes in diverse species included in this application.

Applications of Applicant's discoveries

It will be appreciated while reading the sections that the different experimental molecular genetic approaches focused on different aspects of the pathway from gene and gene product through to the properties of tissues, organs and whole organisms growing in specific environments. For each endogenous gene, these pathways are delineated within the existing biology of the species. However, Applicants' inventions allow gene components or products to be mixed and matched to create new genes and placed in other cellular contexts and species, to exhibit new combinations of functions and characteristics not found in nature, or to enhance and modify existing ones. For instance, gene components can be used to achieve expression of a specific protein in a new cell type to introduce new biochemical activities, cellular attributes or developmental and physiological processes. Such cell-specific targeting can be achieved by combining polynucleotides encoding proteins with any one of a large array of promoters to facilitate synthesis of proteins in a selective set of plant cells. This emphasizes that each gene, component and protein can be used to cause multiple and different phenotypic effects depending on the biological context. The utilities are therefore not limited to the existing in vivo roles of the genes, gene components, and gene products.

While the genes, gene components and products disclosed herein can act alone, combinations are useful to modify or modulate different traits. Useful combinations include different polynucleotides and/or gene components or products that have (1) an effect in the same or similar developmental or biochemical pathways; (2) similar biological activities; (3) similar transcription profiles; or (4) similar physiological consequences.

Of particular interest are the transcription factors and key factors in regulatory transduction pathways, which are able to control entire pathways, segments of pathways or large groups of functionally related genes. Therefore, manipulation of such proteins, alone or in combination is especially useful for altering phenotypes or biochemical activities in plants. Because interactions exist between hormone, nutrition, and developmental pathways, combinations of genes and/or gene products from these pathways also are useful to produce more complex changes. In addition to using polynucleotides having similar transcription profiles and/or biological activities, useful combinations include polynucleotides that may exhibit different transcription profiles but which participate in common or overlapping pathways. Also, polynucleotides encoding selected enzymes can be combined in novel ways in a plant to create new metabolic pathways and hence new metabolic products.



The utilities of the various genes, gene components and products of the Application are described in the sections as follows:

I. Organ Affecting Genes, Gene Components, Products (Including Differentiation Function)

I.A. Root Genes, Gene Components And Products

I.A.1. Root Genes, Gene Components And Products

I.A.2. Root Hair Genes, Gene Components And Products

I.B. Leaf Genes, Gene Components And Products

I.B.1. Leaf Genes, Gene Components And Products

I.B.2. Trichome Genes And Gene Components

I.B.3. Chloroplast Genes And Gene Components

I.C. Reproduction Genes, Gene Components And Products

I.C.1. Reproduction Genes, Gene Components And Products

I.C.2. Ovule Genes, Gene Components And Products

I.C.3. Seed And Fruit Development Genes, Gene Components And Products

I.D. Development Genes, Gene Components And Products

I.D.1. Imbibition and Germination Responsive Genes, Gene Components And Products

I.D.2. Early Seedling Phase Genes, Gene Components And Products

I.D.3. Size and Stature Genes, Gene Components And Products

I.D.4. Shoot-Apical Meristem Genes, Gene Components And Products

I.D.5. Vegetative-Phase Specific Responsive Genes, Gene Components And Products

II. Hormones Responsive Genes, Gene Components And Products

II.A. Abscissic Acid Responsive Genes, Gene Components And Products

II.B. Auxin Responsive Genes, Gene Components And Products

II.C. Brassinosteroid Responsive Genes, Gene Components And Products

II.D. Cytokinin Responsive Genes, Gene Components And Products

II.E. Gibberellic Acid Responsive Genes, Gene Components And Products

5 III. Metabolism Affecting Genes, Gene Components And Products

III.A. Nitrogen Responsive Genes, Gene Components And Products

III.B. Circadian Rhythm Responsive Genes, Gene Components And Products

III.C. Blue Light (Phototropism) Responsive Genes, Gene Components And Products

III.D. Co2 Responsive Genes, Gene Components And Products

10 III.E. Mitochondria Electron Transport Genes, Gene Components And Products

III.F. Protein Degradation Genes, Gene Components And Products

III.G. Carotenogenesis Responsive Genes, Gene Components And Products

15 IV. Viability Genes, Gene Components And Products

IV.A. Viability Genes, Gene Components And Products

IV.B. Histone Deacetylase (Axel) Responsive Genes, Gene Components And Products

V. Stress Responsive Genes, Gene Components And Products

V.A. Cold Responsive Genes, Gene Components And Products

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V.C. Drought Responsive Genes, Gene Components And Products

V.D. Wounding Responsive Genes, Gene Components And Products

V.E. Methyl Jasmonate Responsive Genes, Gene Components And Products

V.F. Reactive Oxygen Responsive Genes, Gene Components And H2O2 Products

25 V.G. Salicylic Acid Responsive Genes, Gene Components And Products

V.H. Nitric Oxide Responsive Genes, Gene Components And Products

V.I. Osmotic Stress Responsive Genes, Gene Components And Products

V.J. Aluminum Responsive Genes, Gene Components And Products

V.K. Cadmium Responsive Genes, Gene Components And Products

30 V.L. Disease Responsive Genes, Gene Components And Products

V.M. Defense Responsive Genes, Gene Components And Products

V.N. Iron Responsive Genes, Gene Components And Products

V.O. Shade Responsive Genes, Gene Components And Products

V.P. Sulfur Responsive Genes, Gene Components And Products

V.Q. Zinc Responsive Genes, Gene Components And Products

5

VI. Enhanced Foods

VII. Pharmaceutical Products

10

VIII. Precursors Of Industrial Scale Compounds

IX. Promoters As Sentinels

SUMMARY OF THE INVENTION

5 The present invention comprises polynucleotides, such as complete cDNA sequences and/or sequences of genomic DNA encompassing complete genes, fragments of genes, and/or regulatory elements of genes and/or regions with other functions and/or intergenic regions, hereinafter collectively referred to as Sequence-Determined DNA Fragments (SDFs) or sometimes collectively referred to as "genes or gene components", or sometimes as "genes, gene components or products", from different plant species, particularly corn, wheat, soybean, rice and *Arabidopsis thaliana*, and other plants and or mutants, variants, fragments or fusions of said SDFs and polypeptides or proteins derived therefrom. In some instances, the SDFs span the entirety of a protein-coding segment. In some instances, the entirety of an mRNA is represented. Other objects of the invention that are also represented by SDFs of the invention are control sequences, such as, but not limited to, promoters. Complements of any sequence of the invention are also considered part of the invention.

10 Other objects of the invention are polynucleotides comprising exon sequences, polynucleotides comprising intron sequences, polynucleotides comprising introns together with exons, intron/exon junction sequences, 5' untranslated sequences, and 3' untranslated sequences of the SDFs of the present invention. Polynucleotides representing the joinder of any exons described herein, in any arrangement, for example, to produce a sequence encoding any desirable amino acid sequence are within the scope of the invention.

15 20 The present invention also resides in probes useful for isolating and identifying nucleic acids that hybridize to an SDF of the invention. The probes can be of any length, but more typically are 12-2000 nucleotides in length; more typically, 15 to 200 nucleotides long; even more typically, 18 to 100 nucleotides long.

25 Yet another object of the invention is a method of isolating and/or identifying nucleic acids using the following steps:

- (a) contacting a probe of the instant invention with a polynucleotide sample under conditions that permit hybridization and formation of a polynucleotide duplex; and
- (b) detecting and/or isolating the duplex of step (a).

30 The conditions for hybridization can be from low to moderate to high stringency conditions. The sample can include a polynucleotide having a sequence unique in a plant genome. Probes and

methods of the invention are useful, for example, without limitation, for mapping of genetic traits and/or for positional cloning of a desired fragment of genomic DNA.

Probes and methods of the invention can also be used for detecting alternatively spliced messages within a species. Probes and methods of the invention can further be used to detect or isolate related genes in other plant species using genomic DNA (gDNA) and/or cDNA libraries. In some instances, especially when longer probes and low to moderate stringency hybridization conditions are used; the probe will hybridize to a plurality of cDNA and/or gDNA sequences of a plant. This approach is useful for isolating representatives of gene families which are identifiable by possession of a common functional domain in the gene product or which have common cis-acting regulatory sequences. This approach is also useful for identifying orthologous genes from other organisms.

The present invention also resides in constructs for modulating the expression of the genes comprised of all or a fragment of an SDF. The constructs comprise all or a fragment of the expressed SDF, or of a complementary sequence. Examples of constructs include ribozymes comprising RNA encoded by an SDF or by a sequence complementary thereto, antisense constructs, constructs comprising coding regions or parts thereof, constructs comprising promoters, introns, untranslated regions, scaffold attachment regions, methylating regions, enhancing or reducing regions, DNA and chromatin conformation modifying sequences, etc. Such constructs can be constructed using viral, plasmid, bacterial artificial chromosomes (BACs), plasmid artificial chromosomes (PACs), autonomous plant plasmids, plant artificial chromosomes or other types of vectors and exist in the plant as autonomous replicating sequences or as DNA integrated into the genome. When inserted into a host cell the construct is, preferably, functionally integrated with, or operatively linked to, a heterologous polynucleotide. For instance, a coding region from an SDF might be operably linked to a promoter that is functional in a plant.

The present invention also resides in host cells, including bacterial or yeast cells or plant cells, and plants that harbor constructs such as described above. Another aspect of the invention relates to methods for modulating expression of specific genes in plants by expression of the coding sequence of the constructs, by regulation of expression of one or more endogenous genes in a plant or by suppression of expression of the polynucleotides of the invention in a plant. Methods of modulation of gene expression include without limitation (1) inserting into a host cell additional

copies of a polynucleotide comprising a coding sequence; (2) modulating an endogenous promoter in a host cell; (3) inserting antisense or ribozyme constructs into a host cell and (4) inserting into a host cell a polynucleotide comprising a sequence encoding a variant, fragment, or fusion of the native polypeptides of the instant invention.

[illegible]

## DETAILED DESCRIPTION OF THE INVENTION

### BRIEF DESCRIPTION OF THE INVENTIONS

As noted above, the Applicants have obtained and analyzed an extensive amount of information on a large number of genes by use of the Ceres Genomic Engine to determine. This information can be categorized into three basic types:

- I. Sequence Information for the Inventions
- II. Transcriptional Information for the Inventions
- III. Phenotypic Information for the Inventions

#### I. Sequence Information

##### Introduction to Sequence Information

To harness the potential of the plant genome, Applicants began by elucidating a large number gene sequences, including the sequences of gene components and products, and analyzing the data. The list of sequences and associated data are presented in the Reference and Sequence Tables of the present application (sometimes referred to as the "REF" and "SEQ" Tables). The Reference and Sequence tables include:

- cDNA sequence;
- coding sequence;
- 5' & 3' UTR;
- transcription start sites;
- exon and intron boundaries in genomic sequence; and
- protein sequence.

The Reference and Sequence Tables also include computer-based, comparative analyses between the protein sequences of the invention and sequences with known function. Proteins with similar sequences typically exhibit similar biochemical activities. The Reference table notes:

- sequences of known function that are similar to the Applicants' proteins; and
- biochemical activity that is associated with Applicants' proteins.

Also, by analyzing the protein sequences, Applicants were able to group the protein sequences into groups, wherein all the sequences in the group contain a signature sequence. The

groups are presented in the Protein Group Table. The signature sequences are reported in the Protein Group Table. More detailed analyses of the signature sequences are shown in the Protein Group Matrix Table.

5                    IA. IDENTIFICATION OF GENE COMPONENTS AND PRODUCTS

To generate the Reference and Sequence Tables, Applicants took a cDNA/coding sequence approach. That is, Applicants initiated their studies either by isolating cDNAs and determining their sequences experimentally, or by identifying the coding sequence from genomic sequence with the aid of predictive algorithms. The cDNA sequences and coding sequences also are referred to as "Maximum Length Sequences" in the Reference tables. The cDNA and coding sequences were given this designation to indicate these were the maximum length of coding sequences identified by Applicants.

Due to this cDNA/coding sequence focus of the present application, the Reference and Sequence Tables were organized around cDNA and coding sequences. Each of these Maximum Length Sequences was assigned a unique identifier: Ceres Sequence ID NO, which is reported in the Tables.

All data that relate to these Maximum Length Sequences are grouped together, including 5' & 3' UTRs; transcription start sites; exon and intron boundaries in genomic sequence; protein sequence, etc.

Below, a more detailed explanation of the organization of the Reference and Sequence Tables and how the data in the tables were generated is provided.

a. cDNA

Applicants have ascertained the sequences of mRNAs from different organisms by reverse transcription of mRNA to DNA, which was cloned and then sequenced. These complementary DNA or cDNA sequences also are referred to as Maximum Length Sequences in the Reference Tables, which contain details on each of the sequences in the Sequence Tables.

Each sequence was assigned a Pat. Appln. Sequence ID NO: and an internal Ceres Sequence ID NO: as reported in the Reference Table, the section labeled "(Ac) cDNA Sequence." An example is shown below:

Max Len. Seq. :



(Ac) cDNA Sequence

- Pat. Appln. Sequence ID NO: 174538

- Ceres Sequence ID NO: 5673127

5 Both numbers are included in the Sequence Table to aid in tracking of information, as shown below:

<210> 174538 (Pat. Appln. Sequence ID NO:)

<211> 1846

<212> DNA (genomic)

<213> Arabidopsis thaliana

<220>

<221> misc\_feature

<222> (1)..(1846)

<223> Ceres Seq. ID no. 5673127

<220>

<221> misc\_feature

<222> ()..()

<223> n is a, c, t, g, unknown, or other

<400> 174538

acaagaacaa caaacagag gaagaagaag aagaagatga agcttctggc tctgtttcca 60

tttctagcga tcgtgatcca actcagctgt... etc.

The Sequence and Reference Tables are divided into sections by organism: *Arabidopsis thaliana*, *Brassica napus*, *Glycine max*, *Zea mays*, *Triticum aestivum*; and *Oryza sativa*.

30 b. Coding Sequence

The coding sequence portion of the cDNA was identified by using computer-based

algorithms and comparative biology. The sequence of each coding sequence of the cDNA is reported in the "PolyP Sequence" section of the Reference Tables, which are also divided into sections by organism. An example shown below for the peptides that relate to the cDNA sequence above

5  
PolyP Sequence

- Pat. Appln. Sequence ID NO 174539
- Ceres Sequence ID NO 5673128
- Loc. Sequence ID NO 174538: @ 1 nt.
- Loc. Sig. P. Sequence ID NO 174539: @ 37 aa.

10  
The polypeptide sequence can be found in the Sequence Tables by either the Pat. Appln. Sequence ID NO or by the Ceres Sequence ID NO: as shown below:

15  
<210> 174539 (Pat. Appln. Sequence ID NO)

<211> 443

<212> PRT

<213> Arabidopsis thaliana

20  
<220>

<221> peptide

<222> (1)..(443)

<223> Ceres Seq. ID no. 5673128

25  
<220>

<221> misc\_feature

<222> ()..()

<223> xaa is any aa, unknown or other

30  
<400> 174539

Thr Arg Thr Thr Lys Gln Arg Lys Lys Lys Lys Lys Met Lys Leu Leu

1            5            10            15

Ala Leu Phe Pro Phe Leu Ala Ile... etc.

25

5

The PolyP section also indicates where the coding region begins in the Maximum Length Sequence. More than one coding region may be indicated for a single polypeptide due to multiple potential translation start codons. Coding sequences were identified also by analyzing genomic sequence by predictive algorithms, without the actual cloning of a cDNA molecule from a mRNA. By default, the cDNA sequence was considered the same as the coding sequence, when Maximum Length Sequence was a spliced together from a genomic annotation.

c. 5' and 3' UTR

The 5' UTR can be identified as any sequence 5' of the initiating codon of the coding sequence in the cDNA sequence. Similarly, the 3' UTR is any sequence 3' of the terminating codon of the coding sequence.

d. Transcription Start Sites

Applicants cloned a number of cDNAs that encompassed the same coding sequence but comprised 5' UTRs of different lengths. These different lengths revealed the multiple transcription start sites of the gene that corresponded to the cDNA. These multiple transcription start sites are reported in the "Sequence # w. TSS" section" of the Reference Tables.

e. Exons & Introns

Alignment of the cDNA sequences and coding portions to genomic sequence permitted Applicants to pinpoint the exon/intron boundaries. These boundaries are identified in the Reference Table under the "Pub gDNA" section. That section reports the gi number of the public BAC sequence that contains the introns and exons of interest.

Max Len. Seq. :

Pub gDNA:

gi No: 1000000005

Gen. seq. in cDNA:

115777 ... 115448 by Method #1  
115105 ... 114911 by Method #1  
114822 ... 114700 by Method #1  
114588 ... 114386 by Method #1  
114295 ... 113851 by Method #1  
115777 ... 115448 by Method #2  
115105 ... 114911 by Method #2  
114822 ... 114700 by Method #2  
114588 ... 114386 by Method #2  
114295 ... 113851 by Method #2  
115813 ... 115448 by Method #3  
115105 ... 114911 by Method #3  
114822 ... 114700 by Method #3  
114588 ... 114386 by Method #3  
114295 ... 113337 by Method #3

(Ac) cDNA Sequence

All the gi numbers were assigned by Genbank to track the public genomic sequences except:

gi 1000000001

gi 1000000002

gi 1000000003

gi 1000000004; and

gi 1000000005.

These gi numbers were assigned by Applicants to the five *Arabidopsis* chromosome sequences that were published by the Institute of Genome Research (TIGR). Gi 1000000001 corresponds to chromosome 1, Gi 1000000002 to chromosome 2, etc.

The method of annotation is indicated as well as any similar public annotations.

f. Promoters & Terminators

Promoter sequences are 5' of the translational start site in a gene; more typically, 5' of the transcriptional start site or sites. Terminator sequences are 3' of the translational terminator codon; more typically, 3' of the end of the 3' UTR.

- 5 For even more specifics of the Reference and Sequence Tables, see the section below titled "Brief Description of the Tables."

## II. TRANSCRIPTIONAL (DIFFERENTIAL EXPRESSION) INFORMATION

### Introduction to Differential Expression Data & Analyses

10 A major way that a cell controls its response to internal or external stimuli is by regulating the rate of transcription of specific genes. For example, the differentiation of cells during organogenesis into forms characteristic of the organ is associated with the selective activation and repression of large numbers of genes. Thus, specific organs, tissues and cells are functionally distinct due to the different populations of mRNAs and protein products they possess. Internal signals program the selective activation and repression programs. For example, internally synthesized hormones produce such signals. The level of hormone can be raised by increasing the level of transcription of genes encoding proteins concerned with hormone synthesis.

15 To measure how a cell reacts to internal and/or external stimuli, individual mRNA levels can be measured and used as an indicator for the extent of transcription of the gene. Cells can be exposed to a stimulus, and mRNA can be isolated and assayed at different time points after stimulation. The mRNA from the stimulated cells can be compared to control cells that were not stimulated. The mRNA levels of particular Maximum Length Sequences that are higher in the stimulated cell versus the control indicate a stimulus-specific response of the cell. The same is true of mRNA levels that are lower in stimulated cells versus the control condition.

20 Similar studies can be performed with cells taken from an organism with a defined mutation in their genome as compared with cells without the mutation. Altered mRNA levels in the mutated cells indicate how the mutation causes transcriptional changes. These transcriptional changes are associated with the phenotype that the mutated cells exhibit that is different from the phenotype exhibited by the control cells.

25 Applicants have utilized microarray and AFLP techniques to measure the levels of mRNAs in cells from mutant plants, stimulated plants, and/or selected from specific organs. The differential

expression of various genes in the samples versus controls are listed in the MA\_diff and/or AFLP\_diff and AFLP\_diff Tables.

Applicants have analyzed the differential data to identify genes whose mRNA transcription levels are positively correlated. From these analyses, Applicants were able to group different genes together whose transcription patterns are correlated. The results of the analyses are reported in the MA\_clust Tables.

a. Experimental Detail

A microarray is a small solid support, usually the size of a microscope slide, onto which a number of polynucleotides have been spotted onto or synthesized in distinct positions on the slide (also referred to as a chip). Typically, the polynucleotides are spotted in a grid formation. The polynucleotides can either be Maximum Length Sequences or shorter synthetic oligonucleotides, whose sequence is complementary to specific Maximum Length Sequence entities. A typical chip format is as follows:

Oligo #1	Oligo #2	Oligo #3
Oligo #4	Oligo #5	Oligo #6
Oligo #7	Oligo #8	Oligo #9

For Applicants' experiments, samples were hybridized to the chips using the "two-color" microarray procedure. A fluorescent dye was used to label cDNA reverse-transcribed from mRNA isolated from cells that had been stimulated, mutated, or collected from a specific organ or developmental stage. A second fluorescent dye of another color was used to label cDNA prepared from control cells.

The two differentially-labeled cDNAs were mixed together. Microarray chips were incubated with this mixture. For Applicants' experiments the two dyes that are used are Cy3, which fluoresces in the red color range, and Cy5, which fluoresces in the green/blue color range. Thus, if:

- cDNA#1 binds to Oligo #1;
- cDNA#1 from the sample is labeled red;
- cDNA#1 from the control is labeled green, and
- cDNA#1 is in both the sample and control,

then cDNA#1 from both the sample and control will bind to Oligo#1 on the chip. If the sample has 10 times more cDNA#1 than the control, then 10 times more of the cDNA#1 would be hybridized to Oligo#1. Thus, the spot on the chip with Oligo#1 spot would look red.

	Oligo #2	Oligo #3
Oligo #4	Oligo #5	Oligo #6
Oligo #7	Oligo #8	Oligo #9

If the situation were reversed, the spot would appear green. If the sample has approximately the same amount of cDNA#1 as the control, then the Oligo#1 spot on the chip would look yellow.

These color differentials are measured quantitatively and used to deduce the relative concentration of mRNAs from individual genes in particular samples.

b. MA\_diff and/or AFLP\_diff Data Table

To generate data, Applicants labeled and hybridized the sample and control mRNA in duplicate experiments. One chip was exposed to a mixture of cDNAs from both a sample and control, where the sample cDNA was labeled with Cy3, and the control was labeled with Cy5 dye. For the second labeling and chip hybridization experiments, the fluorescent labels were reversed; that is, the Cy5 dye for the sample, and the Cy3 dye for the control.

Whether Cy5 or Cy3 was used to label the sample, the fluorescence produced by the sample was divided by the fluorescence of the control. A cDNA was determined to be differentially expressed in response to the stimulus in question if a statistically-significant difference in the sample versus the control was measured by both chip hybridization experiments.

The MA\_diff and/or AFLP\_diff tables show which cDNA were significantly up-regulated as designated by a "+" and which were significantly down-regulated as designated by a "-" for each pair of chips using the same sample and control.

III. PHENOTYPIC INFORMATION

One means of determining the phenotypic effect of a gene is either to insert extra active

copies of the gene or coding sequence, or to disrupt an existing copy of the gene in a cell or organism and measure the effects of the genetic change on one or more phenotypic characters or traits. "Knock-in" is used herein to refer to insertion of additional active copies of a gene or coding sequence. "Knock-out" refers to a plant where an endogenous gene(s) is disrupted. Applicants have used both methods of addition or disruption to determine the phenotypic effects of gene or gene components or products, and have thereby discovered the function of the genes and their utilities.

1. Knock-in results

The coding sequence of a desired protein can be functionally linked to a heterologous promoter to facilitate expression. Here, Applicants have operably linked a number of coding sequences to either one of the promoters listed below:

<u>GFP Pattern</u>	<u>Specific Promoter activity</u>	<u>Plant Line Descriptor</u>
Root epidermis / mostly toward the lower region of root (more intense than CS9094)	Specific to the root basal region.	Root basal
Root-endodermis/cortex (initials sharp); shoot-mesophyll of one leaf, sharp guard cell marking. New leaf petioles near tip of primary inflorescence; floral stems; in flowers at base of sepal, anther stems, and pistil	Specific to the root endodermis-cortex region, leaf petiole, and flowers.	Root/Petiole/Flowers
Broad root exp. (some dermal, some cortical, some vascular); shoot apex. Faintly in petiole; stem	Specific to root and stem.	Root/Stem1
High expression in stem, excluded from 1st true leaves/High in root. Faint expression in stem	Specific to stem and root.	Root/Stem2
Shoot meristem / whole root region; little bit on cotyledons. Base of leaves(axillary meristem?); base of sepals; inflorescence meristem; small amount in unfertilized pistil.	Specific to roots, shoot meristem, base of leaves and flowers.	Root/Stem/Leaves/Flowers
root tip vascular initials; vascular system throughout plant; Bud petal vasculature and pistil septum; Flower petal vasculature; Flower pistil septum; Pre fertilization ovules; Post fertilization ovule at chalazal end; Developing seed (young, maturing siliques); Seed coat and young embryos. GFP not observed in mature embryos.	Specific to vascular systems.	Vascular/Ovule/Young Seed/Embryo



<u>GFP Pattern</u>	<u>Specific Promoter activity</u>	<u>Plant Line Descriptor</u>
Flower, sepal / vascular tissue of root, stem, and cotyledons. Stems of new flowers; vasculature or petals, anthers, sepals, and pistil/silique; Vasculature throughout seedling: root, hypocotyl, petioles, stem, cotyledons, first true leaves; Rosette vasculature; Cauline leaf vasculature; Bud pedicel vasculature; Flower vasculature: (sepals, petals, filaments, pistil); Bud vasculature (sepal, petal, filament, pistil); Funiculus in both flower and bud; Some possible seed coat expression; Silique funiculus; Very faint fluorescence in mature embryo (auto fluorescence perhaps);	Specific to flowers, seed and vasculature.	Flowers/Seed/Vasculature/Embryo
Root expression - primarily in cortex (upper region of the root). No shoot expression	Specific to root.	Roots2
Root expression - less intense in whole root of young seedling. Shoot apical meristem; organ primordia in SAM region.	Specific to root and shoot apical meristem.	Root/SAM
Root epidermis/tip; shoot epidermis/vascular; leaf epidermis; expression in developing seed/ovule - mature embryo; Primary and lateral root cortex; Very strong in root cap; Base of flower bud and epidermis of carpels; Base of flower, epidermis of filaments, epidermis of carpels; Trichomes; Weak (hardly detectable) gfp expression in vasculature throughout seedling; Strong expression in trichomes; POST- fertilization SEED only; GFP strength increases as silique matures; Weak at suspensor end of the embryo; GFP observed in seed coat; Root and post fertilization seed specific gfp expression; Expression in seed coat.	Specific to seed and to epidermal layers of roots, shoots and leaves.	Seed/Epidermis/Ovary/Fruit
Young root dermis; dermal/cortical?/vascular in older root; general (epidermal?) shoot expression; ovules. some in sepals; vasculature of stem	Specific to roots, shoots, and ovules.	Roots/Shoots/Ovule
Vascular tissue of root; Meristem tissues: axillary meristems, floral meristems, base of flowers/sepals; Weak expression in hypocotyl, petiole and cotyledon vasculature..	Specific to root structural leaf vascular region and to floral buds and axillary meristem	Vasculature/Meristem

The chimeric constructs were transformed into *Arabidopsis thaliana*. The resulting transformed lines were screened to determine what phenotypes were changed due to introduced transgene. The phenotype changes, relative to the control, are reported in the Knock-in tables.

5

## 2. Knock-out Results

Knock-out plants in *Arabidopsis thaliana* were created by inserting a polynucleotide tag into the genome. The location of the tag was identified using primers to the tag sequence and isolation of the plant genomic sequence that flanks the tag using a variation of the polymerase chain reaction. The plants were generated using the procedure described in Feldmann et al., (1987) Molec. Gen. Genet. 208: 1-9; Feldmann (1991) Plant Journal, 1:71-83 and Forsthoefel et al., (1992) Aust. J. Plant Physiol. 19:353-366.. On average, the population of plants that was screened had ~1.5 to 2 tags. Generally, the number of tags ranged from 1 to greater than 5.

The polynucleotide tags were classified as either incorporated within a gene, or between two genes. The data in the Knock-out Table indicates which plants have a tag(s) causing a disruption in a gene, or a disruption between genes.

### a. Disruption in a Gene

For the sake of this analysis, the tag was considered to be causing a disruption in a gene when the tag was located:

- 1) less than 501 upstream of the transcriptional start site;
- 2) less than 701 upstream of the translational initiation codon;
- 3) between the translational initiation and termination codons of the gene,
- 4) less than 301 downstream of the translational stop codon; or
- 5) less than 151 downstream of a transcriptional termination site.

By this definition, a tag can be inserted in two genes. For example, if two genes have only 700 nucleotides between the translational termination codon of one gene and the

translational initiation codon of the other gene, the tag can be inserted into the terminator of one gene and the promoter of the other gene according to the definition above.

Genomic annotations by the method OCKHAM-OCDNA identify the transcriptional start and stop site of a gene.

5

b. Disruption between Genes

When a tag causes a disruption between two genes, either or both genes can be affected. Typically, a tag can affect a gene if it disrupts the genome at a location 3000 nt downstream to the start codon of a gene. More typically, insertions found 1000- 2000 nt upstream (5'), or 750-1000 nt downstream (3') could be expected to disrupt expression.

c. More Than One Insert

A plant can have multiple tags. If a mutant phenotype is observed, then it can be attributed to any one or all of the tags.

HOW THE INVENTIONS REVEAL HOW GENES, GENE COMPONENTS AND PRODUCTS  
FUNCTION

The different experimental molecular genetic approaches focused on different aspects of genes, gene components, and gene products of the inventions. The variety of the data demonstrates the multiple functions and characteristics of single genes, gene components, and products. The data also explain the pathways and networks in which individual genes and products participate and interact. As a result, the circumstances or conditions are now known when these genes and networks are active. These new understandings of biology are relevant for many plant species. The following section describes the process by which Applicants analyzed the inventions generated by the Ceres Genomic Engine:

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I. EXPERIMENTAL RESULTS REVEAL MANY FACETS OF A SINGLE GENE

I.A. INTRO: FUNCTIONS AND CHARACTERISTICS OF GENE COMPONENTS AND PRODUCTS ARE IDENTIFIED

The experimental results are used to dissect the function of individual components and products of the genes. For example, the biochemical activity of the encoded protein could be surmised from sequence analyses, and promoter specificity could be identified through transcriptional analyses. Generally, the data presented herein can be used to functionally annotate either the protein sequence and/or the regulatory sequence that control transcription and translation.

I.A.1. FUNCTIONS OF CODING SEQUENCES REVEALED BY THE CERES GENOMIC ENGINE

A.1.a. SEQUENCE SIMILARITY TO PROTEINS OF KNOWN FUNCTION CAN BE USED TO ASSOCIATE BIOCHEMICAL

ACTIVITIES AND MOLECULAR INTERACTION TO THE  
PROTEINS OF THE INVENTION

The protein sequences of the invention were analyzed to determine if they shared any sequence characteristics with proteins of known activity. Proteins can be grouped together based on sequence similarity, either localized or throughout the length of the proteins. Typically, such groups of proteins exhibit common biochemical activities or interact with similar molecules.

1. PRESENCE OF AMINO ACID MOTIFS INDICATES  
BIOLOGICAL FUNCTION

Localized protein sequence similarity, also referred to as amino acid motifs, have been attributed to enzyme or protein functions. A library of motifs, important for function, have been documented in PROSITE, a public database available at <http://www.expasy.ch/prosite/>. This library includes descriptions of the motifs and their functions. The zinc finger motif is one such entry in PROSITE, which reports that the zinc finger domain of DNA-binding proteins is typically defined by a 25-30 amino acid motif containing specific cysteine or histidine residues that are involved in the tetrahedral coordination of a zinc ion. Any protein comprising a sequence similar to the zinc finger amino acid motif will have similar functional activity (specific binding of DNA).

Protein sequences of the invention have been compared to a library of amino acid motifs in the pFAM database, which is linked to the PROSITE database. If any of Applicants' protein sequences exhibit similarity to these amino acid motifs or domains, the Reference Table notes the name and location of the motif in the "Pred. PP Nom. & Annot" section of the Reference tables. A description of any biochemical activities that are associated to these domains, and therefore associated with Applicants' proteins, is included in the Protein Domain table.

For example, polypeptide, CERES Sequence ID NO: 1545823 is associated with zinc finger motif as follows in the Reference Table:

(C) Pred. PP Nom. & Annot.

- Zinc finger, C3HC4 type (RING finger)
- Loc. Sequence ID NO 133059: 58 -> 106 aa.

2. RELATED AMINO ACID SEQUENCES SHARE SIMILAR

BIOLOGICAL FUNCTIONS

It is apparent, when studying protein sequence families, that some regions have been better conserved than others during evolution. These regions are generally important for the function of a protein and/or for the maintenance of its three-dimensional structure.

5 The Reference Table reports in section "(Dp) Rel. AA Sequence" when a protein shares amino acid similarity with a protein of known activity. The section reports the gi number of the protein of known activity, a brief description of the activity, and the location where it shares sequence similarity to Applicants' polypeptide sequence.

Using this analysis, biochemical activity of the known protein is associated with Applicants' proteins. An example for the polypeptide described above is as follows:

(Dp) Rel. AA Sequence

- Align. NO 524716
- gi No 2502079
- Desp. : (AF022391) immediate early protein; ICP0 [Feline herpesvirus 1]
- % Idnt. : 33.7
- Align. Len.: 87
- Loc. Sequence ID NO 133059: 52 -> 137 aa.

10  
15  
20  
A.1.b. DIFFERENTIAL EXPRESSION RESULTS EXPLAIN IN WHICH  
CELLULAR RESPONSES THE PROTEINS OF THE  
INVENTION ARE INVOLVED

Differential expression has been studied in the present instance by both microarray and AFLP approaches. Differential expression results show when the coding sequence is transcribed, and therefore when the activity of the protein is deployed by the cell. Similar coding sequences can have very different physiological consequences because the sequences are expressed at different times or places, rather than because of any differences in protein activity. Therefore, modified levels (increased or decreased) of expression as compared to a control provide an indication of the function of a corresponding gene, gene components, and gene products.

25  
30 These experiments can determine which are genes "over-expressed" under a given stimulus. Such over-expressed genes give rise to higher transcript levels in a plant or cell that is stimulated as



compared to the transcript levels of the same genes in a control organism or cell. Similarly, differential expression experiments can reveal "under-expressed" genes.

To increase the cellular response to a stimulus, additional copies of the coding sequences of a gene that is over-expressed are inserted into a cell. Increasing transcript levels of an over-expressed gene can either heighten or prolong the particular cellular response. A similar enhancement can occur when transcription of an under-expressed gene is inhibited. In contrast, the cellular response will be shortened or less severe when the over-expressed genes are inhibited or when expression of the under-expressed genes are increased.

In addition to analyzing the levels of transcription, the data were also analyzed to gain insight into the changes in transcription over time. That is, while the plants in the experiments were reacting to either an external or internal stimulus, a differential experiment takes a snapshot of the transcription levels in the cells at one specific time. However, a number of snap-shots can be taken at different time points during an external stimulus regime, or at different stages of development during an internal stimulus. These results show how the plant changes transcription levels over time, and therefore protein levels in response to specific stimuli to produce phenotypic changes. These results show that a protein can be implicated in a single, but more likely, in a number of cellular responses.

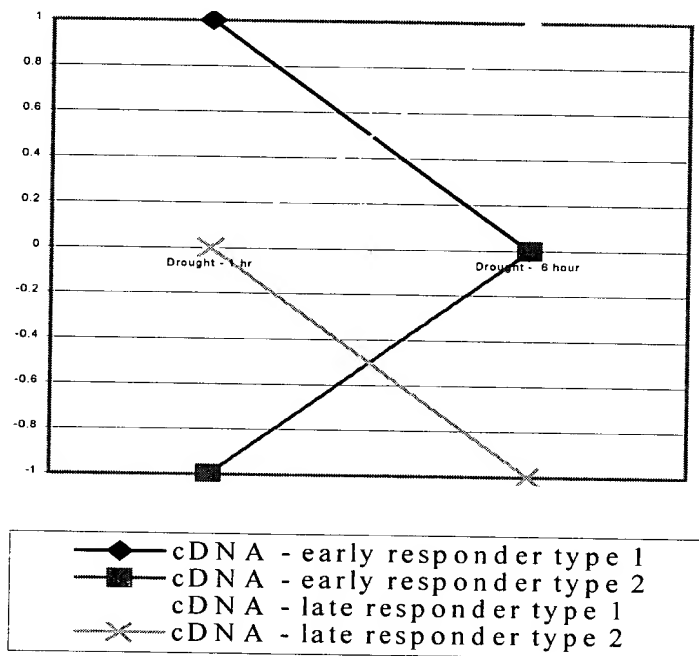
1. THE TRANSCRIPT LEVELS OF A PROTEIN OVER TIME  
IN RESPONSE TO A STIMULI ARE REVEALED BY  
TRANSCRIPTIONAL ANALYSES OVER MANY  
EXPERIMENTS

Applicants produced data from plants at different times after a specific stimulus. These results show whether the expression level of a gene spikes at a key moment during the cellular response, or whether the transcript level remains constant. Thus, coding sequences not only can be determined to be over- or under-expressed, but also can be classified by the initial timing and duration of differential expression. This understanding of timing can be used to increase or decrease any desired cellular response.

Generally, Applicants have assayed plants at 2 to 4 different time points after exposing the plants to the desired stimuli. From these experiments, "early" and "late" responders were identified. These labels are applied to either the regulatory sequences driving transcription of the gene as well

as to the protein encoded by the gene.

The following example illustrates how the genes, gene components and products were classified as either early or late responders following a specific. The mRNAs from plants exposed to drought conditions were isolated 1 hour and 6 hours after exposure to drought conditions. These mRNAs were tested utilizing microarray techniques. The graph below illuminates possible transcription profiles over the time course, plotting all the (+) data points as +1 and all the (-) data points as -1:



(The value for each time point was determined using a pair of microarray chips as described above.)

Data acquired from this type of time course experiment are useful to understand how one may increase or decrease the speed of the cellular response. Inserting into a cell extra copies of the coding sequence of early responders in order to over-express the specific gene can trigger a faster cellular response. Alternatively, coding sequences of late responders that are over-expressed can be placed under the control of promoters of early responders as another means to increase the cellular response.

Inserting anti-sense or sense mRNA suppression constructs of the early responders that are over-expressed can retard action of the late responders, thereby delaying the desired cellular response. In another embodiment, extra copies of the promoters of both early and late responders can be added to inhibit expression of both types of over-expressed genes.

5 The experiments described herein can be grouped together to determine the time course of the transcript levels of different coding sequences in response to different stimuli. Examples of different groups are as follows (the examples include the IDs for both corn and Arabidopsis experiments):

- NAA (EXPT IDs 108564, 108565, 108516, 108554)
- BA (EXPT IDs 108566, 108567, 108517)
- GA (EXPT IDs 108562, 108563, 108519, 108520, 108521, 108484, 108485, 108486)
- BR (EXPT IDs 108580, 108581, 108557, 108478, 108479, 108480, 108481)
- ABA (EXPT IDs 108560, 108561, 108513, 108597)
- Drought (EXPT IDs 108572, 108573, 108502, 108503, 108504, 108556, 108482, 108483, 108473, 108474, 108477)
- Cold (EXPT IDs 108578, 108579, 108533, 108534)
- Heat (EXPT IDs 108576, 108577, 108522, 108523)
- Osmotic stress (EXPT IDs 108570, 108571, 108541, 108542, 108553, 108539, 108540 )
- Reactive Oxygen (EXPT IDs 108582, 108583, 108537, 108538, 108558)
- NO (EXPT IDs 108584, 108585, 108526, 108527, 108559)
- Wounding (EXPT IDs 108574, 108575, 108524, 108525)
- SA (EXPT IDs 108586, 108587, 108515, 108552, 108471, 108472, 108469, 108470, 107953, 107960, 108443, 108440, 108441, 108475, 108476)
- MeJA (EXPT IDs 108568, 108569)
- Finale (EXPT IDs 108467, 107871, 107876)
- Trimec (EXPT IDs 108466, 107886, 107891)
- Round-up (EXPT IDs 108465, 107896)
- Glean (EXPT IDs 108468, 107881)

## 2. THE TRANSCRIPT LEVELS OF A PROTEIN OVER

DIFFERENT DEVELOPMENTAL STAGES CAN BE  
IDENTIFIED BY TRANSCRIPTIONAL ANALYSES OVER  
MANY EXPERIMENTS

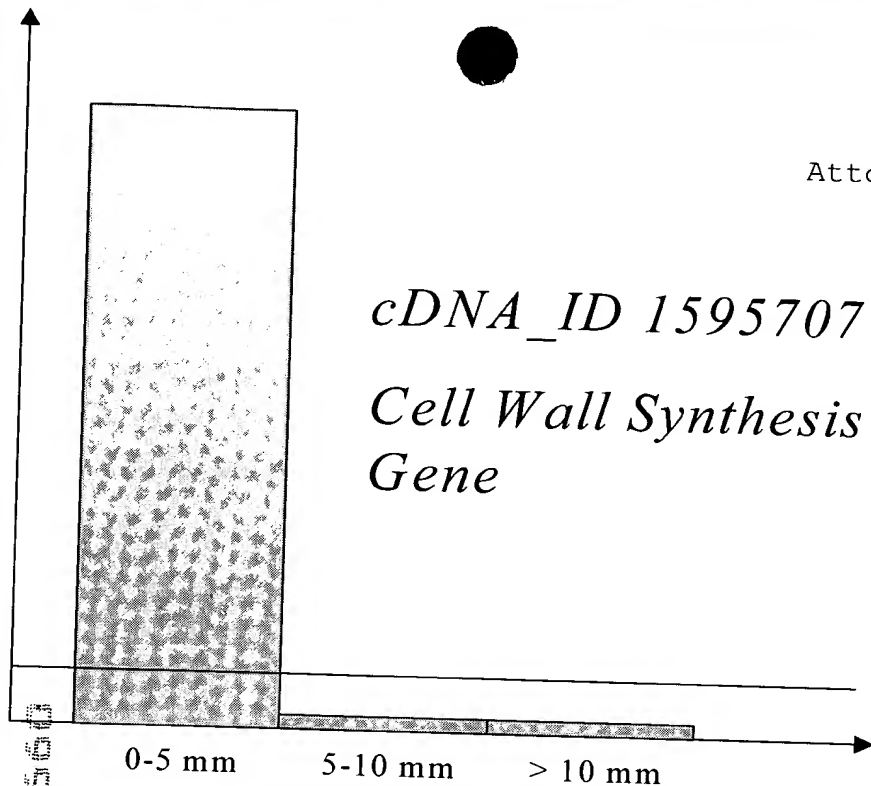
Differential expression data were produced for different development stages of various  
5 organs and tissues. Measurement of transcript levels can divulge whether specific genes give rise to  
spikes of transcription at specific times during development, or whether transcription levels remain  
constant. This understanding can be used to increase speed of development, or to arrest  
development at a specific stage.

Like the time-course experiments, the developmental stage data can classify genes as being  
10 transcribed at early or late stages of development. Generally, Applicants assayed different organs or  
tissues at 2-4 different stages.

Inhibiting under-expressed genes at either early or late stages can trigger faster development  
times. The overall development time also can be increased by this means to allow organs and tissue  
to grow to a larger size or to allow more organs or tissues to be produced. Alternatively, coding  
15 sequences of late stage genes that are under-expressed can be placed under the control of promoters  
of early stage genes to increase heighten development.

Inserting extra copies of the coding sequence early stage genes that are under-expressed can  
retard action of the late-stage genes and delay the desired development.

*cDNA\_ID 1595707*  
*Cell Wall Synthesis*  
*Gene*



Fruit development of *Arabidopsis* is one example that can be studied. Siliques of varying sizes, which are representative of different stages, were assayed by microarray techniques. Specifically, mRNA was isolated from siliques between 0-5mm, between 5-10mm and > 10 mm in length. The graph below shows expression pattern of a cell wall synthesis gene, cDNAID 1595707, during fruit development:

The developmental course shows that the gene encoding a cell wall synthesis protein is up-regulated when the fruit is 0-5mm but returns to normal levels at 5-10mm and > 10mm. Increase of cell wall synthesis can lead to larger cells and/or greater number of cells. This type of increase can boost fruit yield. The coding sequence of the cell wall synthesis protein under the control of a strong early stage promoter would increase fruit size or number.

A pectinesterase gene was also differentially expressed during fruit development, cDNA ID 1396123. Pectinesterase catalyzes the hydrolysis of pectin into pectate and methanol. This biochemical activity plays an important role in cell wall metabolism during fruit ripening. To shorten the time for fruit ripening, extra copies of this gene with its endogenous promoter can be inserted into a desired plant. With its native promoter, the extra copies of the gene will be expressed at the normal time, to promote extra pectinesterase at the optimal stage of fruit development thereby shortening ripening time.

A number of Applicant's experiments can be grouped together to study changes of transcript levels over a number development stages. Below are examples of groups of experiments:

- Root, Root Tip, and rhl mutant (EXPT IDs 108594, 108433, 108599, 108434, 108439)
- Flowers Drought Exposed Flowers, SA Treated Flowers (EXPT IDs 108473, 108474, 108429, 108430, 108431, 108475, 108476, 108501)
- BR Shoot Apices, Leaves, Stm (EXPT IDs 108478, 108479, 108480, 108481, 108598, 108535, 108536, 108435)
- Leaf and Stm (EXPT IDs 108477, 108512, 108497, 108498, 108598, 108478, 108479, 108480, 108481, 108598, 108535, 108536, 108435)
- Imbibed & Germinating Seeds 1, 2, 3, And 4 Days (EXPT IDs 108461, 108462, 108463, 108464, 108528, 108529, 108530, 108531, 108545, 108546, 108547, 108518, 108529, 108543, 108544)
- Tissue Specific Expression (3 week rosette leaves, Tissue Specific Expression (3 week stems), Tissue Specific Expression (2 week roots) (EXPT IDs 108497, 108498, 108439)
- Tissue Specific Expression (3 week rosette leaves), Germinating Seeds (EXPT IDs 108497, 108461)
- Tissue Specific Expression (3 week rosette leaves, stm mutants, BR\_Shoot Apices Expt, root tips, Tissue Specific Expression (2 week roots) (EXPT IDs 108497, 108435, 108480, 108434, 108439)
- BR\_Shoot Apices Expt, root tips, Tissue Specific Expression (flower buds) (EXPT IDs 108480, 108434, 108431)
- Arab\_Ler-pi\_ovule\_1, ap2-floral buds, Tissue Specific Expression (flower buds), Tissue Specific Expression (<5 mm siliques) (EXPT IDs 108595, 108501, 108431, 108437)
- Tissue Specific Expression (2 week roots), rhl mutant2, BR\_Shoot Apices Expt, Trichome Inflorescences (EXPT IDs 108439, 108433, 108480, 108452)

3. PROTEINS THAT ARE COMMON IN A NUMBER OF  
SIMILAR RESPONSES CAN BE IDENTIFIED BY  
TRANSCRIPTIONAL ANALYSES OVER A NUMBER OF

### EXPERIMENTS

The differential expression experiments also reveal the genes, and therefore the coding sequence, that are common to a number of cellular responses. By identifying the genes that are differentially expressed in a number of similar responses, the genes at the nexus of a range of responses are discovered. For example, genes that are differentially expressed in all the stress responses are at the hub of many of the stress response pathways.

These types of nexus genes, proteins, and pathways are differentially expressed in many or majority of the responses or developmental conditions of interest. Typically, a nexus gene, protein, or pathway is differentially expressed in generally the same direction in many or majority of all the desired experiments. By doing so, the nexus gene can be responsible for triggering the same or similar set of pathways or networks for various cellular responses. This type of gene is useful in modulating pleiotropic effects or triggering or inhibiting a general class of responses.

When nexus genes are differentially expressed in a set of responses, but in different directions, these data indicate that a nexus gene is responsible for creating the specificity in a response by triggering the same pathway but to a different degree. Placing such nexus genes under a constitutive promoter to express the proteins at a more constant level can remove the fluctuations. For example, a plant that is better drought adapted, but not cold adapted can be modified to be tolerant to both conditions by placing under the control of a constitutive promoter a nexus gene that is up-regulated in drought but down regulated in cold.

Applicants' experiments can be grouped together to identify such nexus genes. Examples of these groups are as follows:

- Herbicide Response
  - Trimec, Finale, Glean, Round-up (EXPT IDs 108467, 107871, 107876, 108468, 107881, 108465, 107896, 108466, 107886, 107891)
- Stress Response
  - Drought, Cold, Heat, Osmotic Stress (EXPT IDs 108578, 108579, 108533, 108534, 108572, 108573, 108502, 108503, 108504, 108556, 108482, 108483, 108473, 108474, 108477, 108576, 108577, 108522, 108523, 108570, 108571, 108541, 108542, 108553, 108539, 108540)
  - Drought, Cold, Heat, PEG, Trimec, Finale, Glean, Round-up (EXPT IDs 108578,

108579, 108533, 108534, 108572, 108573, 108502, 108503, 108504, 108556,  
108482, 108483, 108473, 108474, 108477, 108576, 108577, 108522, 108523,  
108570, 108571, 108541, 108542, 108553, 108539, 108540)

- Wounding, SA, MeJA, Reactive Oxygen, NO (EXPT IDs 108568, 108569, 108555,  
108584, 108585, 108526, 108527, 108559, 108582, 108583, 108537, 108538,  
108558, 108586, 108587, 108515, 108552, 108471, 108472, 108469, 108470,  
107953, 107960, 108443, 108440, 108441, 108475, 108476, 108574, 108575,  
108524, 108525)

- Hormone Responses

- NAA, BA, BR, GA, TRIMEC (EXPT IDs 108566, 108567, 108517, 108580,  
108581, 108557, 108478, 108479, 108480, 108481, 108562, 108563, 108519,  
108520, 108521, 108484, 108485, 108486, 108564, 108565, 108516, 108554,  
108466, 107886, 107891)
- NAA, Trimec (EXPT IDs 108566, 108567, 108517, 108580, 108581, 108557,  
108478, 108479, 108480, 108481, 108562, 108563, 108519, 108520, 108521,  
108484, 108485, 108486, 108564, 108565, 108516, 108554, 108466, 107886,  
107891)

#### 4. PROTEINS THAT ARE COMMON TO DISPARATE RESPONSES CAN BE IDENTIFIED BY TRANSCRIPTIONAL ANALYSES OVER A NUMBER OF EXPERIMENTS

Phenotypes and traits result from complex interactions between cellular pathways and networks. Which pathways are linked by expression of common genes to specify particular traits can be discerned by identifying the genes that show differential expression of seemingly disparate responses or developmental stages. For example, hormone fluxes in a plant can direct cell patterning and organ development. Genes that are differentially expressed both in the hormone experiments and organ development experiments would be of particular interest to control plant development.

Examples Of Such Pathway Interactions Include:

- (i) The Interaction Between Stress Tolerance Pathways And Metabolism Pathways;



- (ii) Interaction Between Hormone Responses And Developmental Changes In The Plant;
- (iii) Interactions Between Nutrient Uptake And Developmental Changes;
- (iv) Mediation Of Stress Response By Hormone Responses; And
- 5 (v) Interactions Between Stress Response And Development.

Applicant's experiments can be grouped together to identify proteins that participate in interacting pathways or networks. Specific groups of experiments include, for example:

- (i) Stress & Metabolism
  - Germinating Seeds (Day 1), Arab\_0.1uM\_Epi-Brass\_1, Arab\_NO3\_H-to-L\_1, Arab\_100uM\_GA3\_1 (EXPT IDs 108461, 108580, 108592, 108562)
- (ii) Hormones & Development
  - NAA, BA & Root Tips (EXPT IDs 108566, 108567, 108517, 108564, 108565, 108516, 108554, 108434, 108466, 107886, 107891)
  - NAA, Roots & Root Tips (EXPT IDs 108564, 108565, 108516, 108554, 108599, 108434, 108439, 108466, 107886, 107891)
  - NAA, BA, Roots And/Or Root Tips (EXPT IDs 108564, 108565, 108516, 108554, 108599, 108434, 108439, 108466, 107886, 107891, 108566, 108567, 108517)
  - NAA, BA And Leaf (EXPT IDs 108566, 108567, 108517, 108518, 108529, 108512, 108497, 108498, 108598, 108564, 108565, 108516, 108554, 108466, 107886, 107891)
  - NAA, BA, Leaves, Roots And/Or Root Tips (EXPT IDs 108566, 108567, 108517, 108518, 108529, 108512, 108497, 108498, 108598, 108564, 108565, 108516, 108554, 108466, 107886, 107891, 108599, 108434, 108439)
  - ABA & Siliques (Of Any Size) (EXPT IDs 108560, 108561, 108513, 108597, 108436, 108437, 108438)
  - GA, Imbibed & Germinating Seeds, ABA & Siliques (Of Any Size) (EXPT IDs 108560, 108561, 108513, 108597, 108562, 108563, 108519, 108520, 108521, 108484, 108485, 108486, 108461, 108462, 108463,

108464, 108528, 108529, 108530, 108531, 108545, 108546, 108547,  
108518, 108529, 108543, 108544, 108436, 108437, 108438)

- Tissue Specific Expression (3 week rosette leaves), Arab\_0.1uM\_Epi-Brass\_1, Arab\_100uM\_GA3\_1, Germinating Seeds (Day 1), (EXPT IDs 108461, 108497, 108580, 108562, 108461)

(iii) Nutrient Uptake And Development

- Any Or All Nitrogen Experiments With Siliques (Of Any Size) (EXPT IDs 108592, 108593, 108588, 108589, 108590, 108591, 108532, 108548, 108549, 108550, 108551, 108454, 108455, 108487, 108488, 108489, 108436, 108437, 108438)
- Any Or All Nitrogen Experiments With Roots Or Root Tips (EXPT IDs 108518, 108529, 108592, 108593, 108588, 108589, 108590, 108591, 108532, 108548, 108549, 108550, 108551, 108454, 108455, 108487, 108488, 108489, 108594, 108433, 108599, 108434, 108439)

(iv) Stress & Hormones

- ABA, Drought (EXPT IDs 108560, 108561, 108513, 108597, 108572, 108573, 108502, 108503, 108504, 108556, 108482, 108483, 108473, 108474, 108477)
- ABA, Drought, Cold, Heat, & Wounding (EXPT IDs 108560, 108561, 108513, 108597, 108578, 108579, 108533, 108534, 108572, 108573, 108502, 108503, 108504, 108556, 108482, 108483, 108473, 108474, 108477, 108576, 108577, 108522, 108523, 108574, 108575, 108524, 108525)
- Tissue Specific Expression (3 week rosette leaves), Arab\_100uM\_ABA\_1, Ws Arabidopsis Drought 2 days, Ws Arabidopsis Drought 4 days (EXPT IDs 108497, 108560, 108477, 108482)

(v) Stress & Hormones Stress & Hormones

- Nitrogen High transition to Low, Arab\_NO3\_H-to-L\_1, Tissue Specific Expression (<5mm siliques), Tissue Specific Expression (5-10mm siliques) (EXPT IDs 108455, 108592, 108437, 108436)

5

A.1.c. OBSERVATIONS OF PHENOTYPIC CHANGES SHOW WHAT  
PHYSIOLOGICAL CONSEQUENCES APPLICANTS'  
PROTEINS CAN PRODUCE

10 Another direct means of determining the physiological consequences of a protein is to make  
aberrant decreases or increases of its expression level in a cell. To this end, Applicants have  
produced plants where specific genes have been disrupted, or produced plants that include an extra  
expressed copy of the gene. The plants were then planted under various conditions to determine if  
any visible physiological changes are caused. These changes then are attributed to the changes in  
15 protein levels.

I.B. SPECIFICITY OF REGULATORY SEQUENCE ARE UNCOVERED BY THE  
CERES GENOMIC ENGINE

20 I.B.1. DIFFERENTIAL EXPRESSION RESULTS EXPLAIN WHICH  
EXTERNAL OR INTERNAL STIMULI TRIGGER THE REGULATORY  
SEQUENCES

Transcriptional studies can reveal the time and place that genes are expressed. Typically,  
regulatory sequences, such as promoters, introns, UTRs, etc., control when and in which cells  
25 transcription occurs. Differential studies can explain the temporal- and location-specific regulatory  
sequences that control transcription.

Using the experiments that are provided herein, one skilled in the art can choose a promoter  
or any other regulatory sequence that is capable of facilitating the desired pattern of transcription.  
For example, if a promoter is needed to give rise to increased levels of transcription in response to  
30 auxin, but little expression in response to cytokinin, then the promoters of cDNAs that were up-  
regulated in the auxin experiments, but down-regulated the cytokinin experiments would be of

interest.

Time Course Experiments – Time Sensitive

Evaluation of time-course data as described above is also useful to identify time-specific promoters. Promoters or regulatory sequences, like the coding sequences, can be classified as early or late responding according to the microarray data. Promoters that facilitate expression of early or late genes are useful to direct expression of heterologous coding sequences to modulate the cellular response. In the drought data, promoters from “early” responding genes can be selected to activate expression of any desired coding sequence. Thus, a coding sequence for a salt-tolerance protein that is not typically expressed early in response to drought could be linked to an “early” responding promoter to increase salt tolerance within one hour after exposure to drought conditions.

Developmental Experiments – Time Sensitive

Another class of time-sensitive promoters and other regulatory sequence can be identified from the experiments examining different developmental stages. These regulatory sequences can drive transcription of heterologous sequence at particular times during development. For example, expression of stress-responsive genes during fruit development can protect any gain in fruit yield.

Common To Many Pathways – Cause General Effects

Promoters and other regulatory sequence associated with cDNAs that are differentially expressed in a number of similar responses can be used to cause general effects. These types of regulatory sequences can be used to inhibit or increase expression of a desired coding sequence in a number circumstances. For example, protein that is capable of acting as an insecticide can be placed under the control a general “stress” promoter to increase expression, not only when the plant is wounded, but under other stress attack.

II. EXPERIMENTAL RESULTS ALSO REVEAL PATHWAYS OR NETWORKS OF GENES

II.A. GENES WHOSE TRANSCRIPTION ARE WELL COORDINATED  
GENERALLY ACT TOGETHER TO PRODUCE PROTEINS THAT

PARTICIPATE IN THE SAME PATHWAY OR NETWORK

Patrick Brown, one of the pioneers of microarray chip technology, demonstrated that differential expression experiments can identify groups of genes that encode proteins that participate the same pathway or network. The work focused on phosphate accumulation and metabolism genes in yeast and was published in the paper Ogawa *et al.*, Mol Biol Cell (2000) Dec;11(12):4309-21. The authors identified by microarray analysis 22 genes whose transcription was regulated by phosphate concentration. Promoter analysis of these genes showed that 21 of them contained a sequence in their promoters that is recognized by a transcriptional activator that is regulated by phosphate. Further, phenotypic studies were completed by mutational analysis of many of these 22 genes in yeast. The mutants were shown to be either severely deficient in accumulation of inorganic polyphosphate (polyP) and P(i), or associated with normal catabolism of polyP in the yeast vacuole. This publication proves that genes with correlated transcriptional profiles do indeed participate in the same pathway or network.

II.A.1. CALCULATING THE CORRELATION COEFFICIENT BETWEEN  
PAIRS OF GENES BASED ON THE DIFFERENTIAL EXPRESSION  
DATA

The differential expression data obtained over many experiments reveal the global pattern of transcription of a gene. Transcription patterns, also referred to as profiles, of two different genes can be compared. From this comparison, a correlation coefficient can be calculated as a measure of the strength of the relationship between the two profiles.

Transcription profiles can be compared by plotting as a point, the differential expression of gene1 on the x-axis and gene 2 on the y-axis on one experiment. If all the pairs lie on a regression line the relationship and correlation between the two genes are strong. The correlation coefficient can be calculated using a number of methods. In the present case, the Spearman method was utilized.

The correlation coefficient can vary from -1 to 1. The coefficient indicates the strength of the relationship between two mRNA transcripts of any set of data that is examined. A zero coefficient indicates that no correlation exists between the transcription profiles of two genes in the samples examined.

Biologically, a high correlation coefficient indicates that a gene(s) triggers the activation or repression of the correlated genes, or have related functional roles. Thus, illumination of the activity of one gene can indicate the activities of the genes with highly correlated transcription profiles. This implication is true whether the activity is a biochemical activity, molecular interaction, cellular response, or physiological consequence.

II.A.2. THE COMPLETE LINKAGE ANALYSES OF DIFFERENTIAL  
IDENTITY GENES WITH SIMILAR PATTERN OF TRANSCRIPTION

The complete linkage analysis can build groups (or "clusters") of genes whose transcription patterns are highly correlated or co-regulated.

Because genes with related functions are frequently expressed in similar patterns, utilities or roles can be ascribed for genes (without observation of transformed plants) based on their temporal association with other genes of known function (a "guilt-by-association" analysis). Ogawa *et al.* has used correlated mRNA transcription profiles to identify the function of proteins of unknown function.

The complete linkage analysis utilizes the correlation coefficients that are calculated for each pair of genes tested in the microarray experiments. A cluster is first seeded with any arbitrary transcript tested on the chip. The seed transcript, for this illustration, is designated mRNA#0. Next, a minimum threshold is chosen for all acceptable correlation coefficients. In this case, the threshold used was 0.75. A list of potential cluster members is compiled by choosing mRNA transcripts that have a correlation coefficient with mRNA#0 that is greater than the threshold. No limit is placed on the number of mRNAs that can be added to a cluster so long as the correlation coefficient meets the threshold limit criterion.

For this example, assume that four mRNAs were added to the cluster, mRNA\_1 to mRNA\_4. Once the potential cluster members are identified, the cDNA IDs of each member is added to the potential list in order its correlation coefficient to mRNA#1, the largest correlation coefficient first. For this example, let's suppose four mRNAs 1-4 are potential members, they would be ordered as follows:

MRNA#	Correlation Coefficient with mRNA#0

MRNA#1	0.9
MRNA#2	0.8
MRNA#3	0.78
MRNA#4	0.75

A potential member is accepted into the group, if its correlation coefficients with all other potential members are all greater than the threshold. Thus, for mRNA#1 to remain in the group the correlation coefficient between mRNA#1 and mRNA#2 must be greater than 0.75; and mRNA#1 and #3 > 0.75; and mRNA#1 and mRNA#4 > 0.75. Potential cluster members are removed only after reviewing the correlation coefficients in a specific order where mRNAs are reviewed in the order that they appear on the list.

Consequently, review of the correlation coefficients does not begin with any random pair, such as mRNA#3 and mRNA#4. The review begins between mRNA#1 and mRNA#2, which are the top two on the list.

If correlation coefficient between mRNA#1 and mRNA#2 is less than the threshold, then mRNA#2 is removed from the cluster. mRNA#2 is removed because its correlation coefficient with mRNA#0 is 0.8 which is less than 0.9, the correlation coefficient of mRNA#1 and mRNA#0.

This illustrates the rule that if the correlation coefficient is less than the threshold, then only one of the pair not accepted as a cluster member, specifically, the one with the lower coefficient to the seed mRNA#0.

This process of iterative reviewing of correlation coefficients between potential members continues until all pairs are reviewed. In this case, the coefficient between mRNA#1 and mRNA#3 would be reviewed because these are the two highest ones on the list besides mRNA#1 and #2. The next pair to be reviewed would be mRNA#1 and #4, etc.

Applicants have analyzed the data using several sets of parameters for the complete linkage analysis as shown in the table below:

Method	Correlation Coefficient Threshold	Max number of members in a cluster	Organism
CL_METHOD_TYPE=	0.9	MAX_SIZE=15	Arabidopsis

TRUE			
CL_METHOD_TYPE=TRUE	0.75	MAX_SIZE=30000	Arabidopsis
CL_METHOD_TYPE=TRUE	0.70	MAX_SIZE=30000	Arabidopsis
CL_METHOD_TYPE=TRUE	0.9	MAX_SIZE=15	Zea
CL_METHOD_TYPE=TRUE	0.75	MAX_SIZE=30000	Zea
CL_METHOD_TYPE=TRUE	0.70	MAX_SIZE=30000	Zea
CL_METHOD_TYPE=TRUE	0.9	MAX_SIZE=15	Arabidopsis
CL_METHOD_TYPE=TRUE	0.75	MAX_SIZE=30000	Arabidopsis
CL_METHOD_TYPE=TRUE	0.70	MAX_SIZE=30000	Arabidopsis
CL_METHOD_TYPE=TRUE	0.9	MAX_SIZE=15	Zea
CL_METHOD_TYPE=TRUE	0.75	MAX_SIZE=30000	Zea
CL_METHOD_TYPE=TRUE	0.70	MAX_SIZE=30000	Zea

The results of these cluster analyses are reported in the MA\_clust table.

### II.A.3. THE NEAREST NEIGHBOR ANALYSES OF DIFFERENTIAL GROUP GENES WITH CORRELATED BUT DISSIMILAR TRANSCRIPTION PROFILES

The nearest neighbor analysis differs from the complete linkage algorithm by not requiring all members to meet the correlation threshold with each other. Thus, a member of a nearest neighbor cluster need only be closely correlated to one other member of the cluster. It is not even required that all members be closely correlated to the seed mRNA transcript.

In a complete linkage cluster all the transcription profile of all members are correlated to a greater or lesser extent. In contrast, a cluster deduced by the nearest neighbor analysis may include members with differing transcription profiles. However, nearest neighbor brings to light



clusters of interacting genes. In the nearest neighbor analysis, the seed mRNA may not have a very high correlation coefficient with the last mRNA added to the cluster.

The nearest neighbor analysis, like the complete linkage analysis, is initiated by seeding each cluster with a mRNA\_0. The cluster size is determined by setting a threshold coefficient and setting a limit on the number of members that can be added to the cluster.

The cluster is expanded in an iterative fashion determining which mRNA has the highest correlation coefficient with mRNA\_0. The additional member is labeled mRNA\_1. Next, a list of potential candidates is generated by finding the mRNA that has the highest correlation to mRNA\_0 (besides mRNA\_1) and finding the mRNA that has the highest coefficient with mRNA\_1. Whichever of the candidates has the highest correlation coefficient is added to the cluster. Then, a list of three potential candidates is generated similarly.

Addition of members continues until either (1) all the correlation coefficients of potential members is lower than the threshold or (2) number of members in the cluster meets the size limitation.

Applicants have analyzed the data using several sets of parameters for the nearest neighbor analysis as shown in the table below:

Method	Correlation Coefficient Threshold	Max number of members in a cluster	Organism
NN_METHOD_TYPE=TRUE	0.5	MAX_HITS=15	Arabidopsis
FULL_NN_METHOD_TYPE=TRUE	0.8	NONE	Arabidopsis
FULL_NN_METHOD_TYPE=TRUE	0.6	NONE	Arabidopsis
NN_METHOD_TYPE=TRUE	0.5	MAX_HITS=15	Zea
FULL_NN_METHOD_TYPE=TRUE	0.8	NONE	Zea
FULL_NN_METHOD_TYPE=TRUE	0.6	NONE	Zea

NN_METHOD_TYPE=TRUE	0.5	MAX_HITS=15	Arabidopsis
FULL_NN_METHOD_TYPE=TRUE	0.8	NONE	Arabidopsis
FULL_NN_METHOD_TYPE=TRUE	0.6	NONE	Arabidopsis
NN_METHOD_TYPE=TRUE	0.5	MAX_HITS=15	Zea
FULL_NN_METHOD_TYPE=TRUE	0.8	NONE	Zea
FULL_NN_METHOD_TYPE=TRUE	0.6	NONE	Zea

The results of these cluster analyses are reported in the MA\_clust table.

### III. EXPERIMENTAL RESULTS REVEAL THE FUNCTIONS AND CHARACTERISTICS OF GENES, PATHWAYS AND NETWORKS

#### III.A. LINKING BIOCHEMICAL OR METABOLIC ACTIVITIES OF ONE PROTEIN IN A CLUSTER TO THE OTHER PROTEINS IN THE SAME MICROARRAY CLUSTER

As shown in the Ogawa *et al.*, Mol Biol Cell (2000), genes whose transcription profiles cluster together as being strongly correlated typically take part in the same pathway or network. Thus, the activity of one gene in the cluster can be associated to the other genes in the cluster with highly correlated transcription profiles. This association is true whether the activity is a biochemical activity, molecular interaction, cellular response or physiological consequence.

One example of this is cluster 420 of the report (shown below). In this cluster, a protein encoded by cDNA ID 1025791 did not match to any pFAM domain. However, through the microarray data, the gene that encodes that protein had a transcription profile that was correlated with other genes that encode ribosomal proteins. Thus, the activity of the ribosomal genes can be associated with the protein with no pFAM match. All the proteins in the same cluster would be associated with mRNA translation and protein synthesis.

420	1025791	803433	4585878	(AC005850) Unknown protein [Arabidopsis]		
420	4608965	671877	8567795	(AC013428) 40S ribosomal protein S17, pu	Ribosomal _S17e	Ribosomal S17
420	5663116	818554	7486478	hypothetic al protein F6E13.17 - Arabidop	DapB	Dihydrodi picolinate reductase

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### III.B. USING DIFFERENTIAL EXPRESSION DATA TO DETERMINE WHEN THE GENES AND PATHWAYS ARE ACTIVE

The differential expression data can be used to associate the cellular response that results when the clusters of genes are transcribed. For the complete linkage clusters, the genes in the cluster will produce similar transcription profiles. The experiments where the genes in the cluster are differentially expressed as compared to the control define the cellular responses that all the genes of the cluster are capable of modulating.

For example, for the cluster shown above, the mRNA levels for the genes were significantly different in the nitrogen response experiments. Thus, the data shows that this cluster of genes is associated with protein synthesis in response to nutrient uptake.

5           III.C. USING PHENOTYPE DATA TO DETERMINE WHEN GENES AND  
              PATHWAYS ARE ACTIVE

10           The phenotypic data can be used to demonstrate the physiological consequences of that  
              result when a cluster of genes is active. Whether the clusters were generated by the complete  
              linkage or the nearest neighbor analyses, if a single gene in the cluster has been implicated in  
              phenotypic changes, then any one or combination of the other genes in the cluster can also modulate  
              the same or similar phenotypic changes.

Utilities of Particular Interest

15           The following sections describes utilities/functions for the genes, gene components and  
              products of the invention. The sequences of the invention, as discussed above, can be recognized as  
              a particular type of gene (e.g. root gene, leaf gene, etc.) by means of particular terms utilized in the  
              Knock-in and Knock-out Tables and by the results of the differential expression experiments.  
              Combined analysis of those data also identify genes with utilities/functions of particular interest.  
              The Single Gene Functions and Utilities Table correlates that data and specific genes with those  
20           utilities/functions of particular interest.

Utilities of Particular Interest for Clustered Sequences

25           As discussed further herein, the genes, gene components and products of the invention have  
              been clustered together into groups. This enables one to understand the function/utility of one  
              member of the cluster based upon knowledge about one or more other members of the cluster. In  
              addition, this enables an understanding of some utilities/functions of a cluster that would be of  
              particular interest. The Cluster Functions and Utilities Table lists some of the clusters of the  
              invention and notes the functions/utilities that are of particular interest for each of the clusters. Of  
              course, these functions/utilities are of particular interest for each member of each particular cluster.

30           IV. EXPERIMENTAL RESULTS PROVIDE AN UNDERSTANDING OF GENES,

PATHWAYS AND NETWORKS IN MANY PLANT SPECIES

IV.A. INTRO: USING PROTEIN Sequence SIMILARITY TO ASSIGN FUNCTION  
FROM ARABIDOPSIS PROTEINS TO PROTEINS OF OTHER SPECIES

5 By analyzing the constant and variable properties of groups of similar sequences, it is possible to derive a structural and functional signature for a protein family, which distinguishes its members from all other proteins. This approach has allowed the Applicants to assign proteins into functional groups and identify orthologous proteins both within and between species. A pertinent analogy to be considered is the use of fingerprints by the police for identification purposes. A fingerprint is generally sufficient to identify a given individual. Similarly, a protein signature can be used to assign a newly sequenced protein to a specific family of proteins and thus to formulate hypotheses about its function.

10 Proteins can be grouped together because they share a single motif or many motifs. Typically, proteins that share a series of motifs share greater functional equivalence. Usually, signature sequences comprise more than one motif in a particular order from N-terminus to C-terminus.

15 A list of these groups can be found in the Protein Group Table. The sequences were grouped together using the iterative protein sequence local alignment software, PSI-BLAST. This software begins by aligning a number sequences where the probability that the alignment occurred by chance is set by a threshold e-value. In the Applicants' case, the threshold e-value was set at  $10^{-50}$ ,  $10^{-30}$ , and  $10^{-10}$ . The algorithm generates a consensus sequence from the sequences that were aligned together. The consensus sequence was then used to find sequences that matched to it with a probability that was less than the set threshold. The algorithm performs the iterative tasks of aligning and generating a consensus sequence any number of times. Generally, Applicants performed one iteration for the  $10^{-10}$  e-value threshold, two iterations for the  $10^{-30}$  threshold, and three iterations for the  $10^{-50}$  threshold.

20 Each group can contain sequences from one of more organisms. The groups included both Ceres polypeptides and public polypeptide sequences. The Ceres polypeptides are identified by their Ceres Sequence ID NO as listed in the Reference Table.

25 Each group contains sequences that were included at the  $10^{-50}$ ,  $10^{-30}$ , and  $10^{-10}$  e-value cutoffs. For each group, the peptide ID and at which cutoff the peptide was included into the group.

The same peptide ID may be included in the group three times as peptide ID 50, peptide ID 30 and peptide ID 10. The data indicates that peptide ID was included in the group when the threshold was either  $10^{-50}$ ,  $10^{-30}$ , or  $10^{-10}$ . All the peptide IDs that are followed by "50" were included in the protein group when the e-value cutoff was  $10^{-50}$ . All the peptide IDs that are followed by either "30" or "50" were included in the protein group when the threshold e-value was  $10^{-30}$ . All the peptide IDs that are followed by "10", "30" or "50" were included in the protein group when  $10^{-10}$  was used as the e-value cutoff.

#### IV.A.1. CONSERVED SEQUENCES BETWEEN PROTEINS OF DIFFERENT SPECIES GIVE RISE TO A SIGNATURE SEQUENCE

The signature sequence for each group of proteins, also referred to as the consensus sequence. The signature sequence comprises the amino acids that are conserved throughout all the proteins in a particular protein group. The data are shown in the Protein Group table.

Not all the polypeptides in a group are the same length. Thus, some members of the group may not contain the entire signature sequence. However, throughout the length of any member protein, its sequence will match the signature sequence.

The consensus sequence contains both lower-case and upper-case letters. The upper-case letters represent the standard one-letter amino acid abbreviations. The lower case letters represent classes of amino acids:

- "t" refers to tiny amino acids, which are specifically alanine, glycine, serine and threonine.
- "p" refers to polar amino acids, which are specifically, asparagine and glutamine
- "n" refers to negatively charged amino acids, which are specifically, aspartic acid and glutamic acid
- "+" refers to positively charged residues, which are specifically, lysine, arginine, and histidine
- "r" refers to aromatic residues, which are specifically, phenylalanine, tyrosine, and tryptophan,
- "a" refers to aliphatic residues, which are specifically, isoleucine, valine, leucine, and methionine

In addition to each consensus sequence, Applicants have generated a scoring matrix to provide further description of the consensus sequence. The matrix reports the identity and number of occurrences of all the amino acids that were found in the group members for every residue position of the signature sequence. The matrix also indicates for each residue position, how many different organisms were found to have a polypeptide in the group that included a residue at the relevant position. These results are reported in the Protein Group Matrix table.

Functional equivalents share similar (1) structural characteristics; (2) biochemical activities and molecular interactions; (3) cellular responses or activities; or (4) phenotypic effects.

#### IV.A.2.LINKING SIGNATURE SEQUENCES TO CONSERVATION OF STRUCTURAL CHARACTERISTICS

Proteins with related functions show similar three-dimensional structures but may not show extensive amino acid sequence similarity. Typically, proteins need only share a single motif or low similarity in multiple domains to exhibit similar structural features, such as alpha helix, beta sheet, charge residues, stretches of hydrophobicity, etc. Conserved structural features have been implicated in ligand binding by receptor proteins, binding to a class of substrates, polynucleotide binding, or protein-protein interactions.

Based on the signature sequences and the Matrix Tables described herein, a number of motifs can be discerned. Motifs are identified as regions in the signature sequence which are constant in a majority of the members of the group. Example motifs can be found among Applicant's data which are shared in the range of 75% to 95% of group members.

Typically, a region of the consensus sequence is constant if, at each position of the region, the preferred amino acid is chosen from a single class of amino acids; even more typically, the preferred amino acid is a single amino acid. The region can contain a number of positions where an amino acid can be chosen. However, these variable positions are usually less than 15% of the total number of residues in the region; more usually, less than 10%; even more usually, less than 5%.

Generally, a domain is considered to be well defined if the consensus sequence is constructed from sequences from at least 2 organisms; more preferably, at least 3 organisms; even more preferably four organisms or greater.

Primary domains are best identified from the data presented for the  $10^{-10}$  probability criteria.

Using this parameter, the largest number of proteins is associated into a group. Consequently, the signature sequence exhibits the greatest amount of variability. The conserved regions, the domains or motifs of the signature contrast against the variable regions. These variable regions become obvious when sequences from more proteins are compared.

5           Signature sequences revealed in the  $10^{-30}$  and  $10^{-50}$  e-value classes show more conservation in the domains, and can even display a degree of conservation in what is considered the variable regions in the  $10^{-10}$  analyses. These more extensively-conserved domains can reflect higher similarity in function – completely orthologous functions. Proteins that share a number of conserved domains, in the same relative order from N terminus to C terminus, are even more likely  
10 to be completely orthologous. Nevertheless, because of the natural divergence that occurs in non-conserved regions during evolution and species differentiation, orthologs can be proteins with only the domains conserved and therefore be present in the  $10^{-30}$  and  $10^{-10}$  p value classes of the Ortholog Table.

#### IV.A.3.LINKING SIGNATURE SEQUENCES TO CONSERVATION OF BIOCHEMICAL ACTIVITIES AND MOLECULAR INTERACTIONS

Proteins that possess the same defined domains or motifs are likely to carry out the same biochemical activity or interact with a similar class of target molecule, e.g., DNA, RNA, proteins, etc. Thus, the pFAM domains listed in the Reference Tables are routinely used as predictors of  
20 these properties. Substrates and products for the specific reactions can vary from protein to protein. Where the substrates, ligands, or other molecules bound are identical the affinities may differ between the proteins. Typically, the affinities exhibited by different functional equivalents varies no more than 50%; more typically, no more than 25%; even more typically, no more than 10%; or even less.

25           Proteins with very similar biochemical activities or molecular interactions will share similar structural properties, such as substrate grooves, as well as sequence similarity in more than one motif. Usually, the proteins will share at least two motifs of the signature sequence; more usually, three motifs; even more usually four motifs or greater. Typically, the proteins exhibit 70% sequence identity in the shared motifs; more typically, 80% sequence identity; even more typically,  
30 90% sequence identity or greater. These proteins also often share sequence similarity in the variable regions between the constant motif regions. Further, the shared motifs will be in the same order



from amino- to carboxyl-termini. The length of the variable regions between the motifs in these proteins, generally, is similar. Specifically, the number of residues between the shared motifs in these proteins varies by less than 25%; more usually, does not vary by less than 20%; even more usually, less than 15%; even more usually less than 10% or even less.

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#### IV.A.4.LINKING SIGNATURE SEQUENCES TO CONSERVATION OF CELLULAR RESPONSES OR ACTIVITIES

Proteins that exhibit similar cellular response or activities will possess the structural and conserved domain/motifs as described in the Biochemical Activities and Molecular Interactions above.

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Proteins can play a larger role in cellular response than just their biochemical activities or molecular interactions suggest. A protein can initiate gene transcription, which is specific to the drought response of a cell. Other cellular responses and activities include: stress responses, hormonal responses, growth and differential of a cell, cell to cell interactions, etc.

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The cellular role or activities of protein can be deduced by transcriptional analyses or phenotypic analyses as well as by determining the biochemical activities and molecular interactions of the protein. For example, transcriptional analyses can indicate that transcription of gene A is greatly increased during flower development. Such data would implicate protein A encoded by gene A, in the process of flower development. Proteins that shared sequence similarity in more than one motif would also act as functional equivalents for protein A during flower development.

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#### IV.A.5.LINKING SIGNATURE SEQUENCES TO CONSERVATION OF PHENOTYPIC EFFECTS

Typically, proteins that are grouped together under the most stringent parameters, e-value  $\leq 10^{-50}$ , are likely orthologs and therefore, when present in the same or equivalent cells can cause similar phenotypic consequences. These proteins have very high sequence similarity. Typically, if one of the members of a group is an *Arabidopsis* protein, then the corn ortholog can rescue an *Arabidopsis* mutant plant that does not produce the *Arabidopsis* protein. The mutant plant would be rescued as the parental "wild-type" phenotype by expression of a coding sequence of the corn protein of the same orthologous group when present in the appropriate cell types of the plant.

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Preferably, these functional equivalents have sequence motif identity throughout much of

the length of the protein. However, proteins that share very high similarity between a number, usually more than two; even more usually, more than three motifs can act as functional equivalents to produce similar phenotypic effects.

5 A gene can have coding sequence similarity, i.e., is a homologous. The coding sequence can be sufficient to act as a functional equivalent, although the gene as a whole is not an ortholog. For example, two similar dwf4 coding sequences were found in the *Arabidopsis* genome. However, this pair of coding sequences had different promoters and hence different roles in *Plantae*. But when one of the pair was placed under the control of its mates' promoter, the phenotypic effects were similar to the effects produced by its mate coding sequence. Therefore, the coding sequence, 10 but not the genes are orthologous.

## APPLICATIONS OF THE INVENTIONS

As described herein, the results of Applicant's experiments provide an understanding of the function and phenotypic implications of the genes, gene components and products of the present invention. Bioinformatic analysis provides such information. The sections of the present application containing the bioinformatic analysis, together with the Sequence and Reference Tables, teach those skilled in the art how to use the genes, gene components and products of the present invention to provide plants with novel characteristics. Similarly, differential expression analysis provides additional such information and the sections of the present application on that analysis; together with the MA\_diff and/or AFLP\_diff Tables and MA\_Cluster Tables, describe the functions of the genes, gene components and products of the present invention which are understood from the results of the differential expression experiments. The same is true with respect to the phenotype data, wherein the results of the Knock-in and Knock-out experiments and the sections of the present application on those experiments provide the skilled artisan with further description of the functions of the genes, gene components and products of the present invention.

As a result, one reading each of these sections of the present application as an independent report will understand the function of the genes, gene components and products of the present invention. But those sections and descriptions can also be read in combination, in an integrated manner, to gain further insight into the functions and uses for the genes, gene components and products of the present invention. Such an integrated analysis does not require extending beyond the teachings of the present application, but rather combining and integrating the teachings depending upon the particular purpose of the reader.

Some sections of the present application describe the function of genes, gene components and products of the present invention with reference to the type of plant tissue (e.g. root genes, leaf genes, etc.), while other sections describe the function of the genes, gene components and products with respect to responses under certain conditions (e.g. auxin-responsive genes, heat-responsive genes, etc.). Thus, if one desires to utilize a gene understood from the application to be a particular tissue-type of gene, then the condition-specific responsiveness of that gene can be understood from the differential expression tables, and very specific characteristics of actions of that gene in a transformed plant will be understood by recognizing the overlap or intersection of the gene

functions as understood from the two different types of information. Thus, for example, if one desires to transform a plant with a root gene for enhancing root growth and performance, one can know the useful root genes from the results reported in the knock-in and knock-out tables. A review of the differential expression data may then show that a specific root gene is also over-expressed in response to heat and osmotic stress. The function of that gene is then described in (1) the section of the present application that discusses root genes, (2) the section of the present application that discusses heat-responsive genes, and (3) the section of the application that discusses osmotic stress-responsive genes. The function(s) which are commonly described in those three sections will then be particularly characteristic of a plant transformed with that gene. This type of integrated analysis of data can be viewed from the following schematic that summarizes, for one particular gene, the function of that gene as understood from the phenotype and differential expression experiments.

Gene function known from phenotype experiments	Gene function known from first differential expression experiment	Gene function known from second differential expression experiment
Function A	Function A	Function A
Function B		
	Function C	Function C
	Function D	
		Function E
Function F	Function F	Function F
Function G	Function G	
		Function H
Function I		Function I
	Function J	

In the above example, one skilled in the art will understand that a plant transformed with this particular gene will particularly exhibit functions A and F because those are the functions which are

understood in common from the three different experiments.

Similar analyses can be conducted on various genes of the present invention, by which one skilled in the art can effectively modulate plant functions depending upon the particular use or conditions envisioned for the plant.

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I. ORGAN-AFFECTING GENES, GENE COMPONENTS, PRODUCTS (INCLUDING  
DIFFERENTIATION AND FUNCTION)

5 I.A. ROOT GENES, GENE COMPONENTS AND PRODUCTS

I.A.1. ROOT GENES, GENE COMPONENTS AND PRODUCTS

10 The economic values of roots arise not only from harvested adventitious roots or tubers,  
but also from the ability of roots to funnel nutrients to support growth of all plants and increase  
their vegetative material, seeds, fruits, etc. Roots have four main functions. First, they anchor  
the plant in the soil. Second, they facilitate and regulate the molecular signals and molecular  
traffic between the plant, soil, and soil fauna. Third, the root provides a plant with nutrients  
gained from the soil or growth medium. Fourth, they condition local soil chemical and physical  
properties.

a. Identification of Root Genes

Root genes identified herein are defined as genes, gene components and products capable  
of modulating one or more processes in or functions of the root as described below. They are  
active or potentially active to a greater extent in roots than in most other organs of the plant.  
20 These genes and gene products can regulate many plant traits from yield to stress tolerance. That  
single genes usually affect the development and function of roots and whole plants is a  
consequence of biological cellular complexity and the role roots play in supporting the growth of  
whole plants. Examples of such root genes and gene products are shown in the Reference and  
Sequence Reference and Sequence Tables and sequences encoding polypeptides of the Protein  
25 Group and Protein Group Matrix tables or fragments thereof, the Knock-In and Knock-Out  
Tables, and the MA-diff Tables. The function of many of the protein products gained from  
comparisons with proteins of known functions, are also given in the REF Tables.

Root Genes Identified By Phenotypic Observations

30 Root genes are active or potentially active to a greater extent in roots than in some other  
organs/tissue of the plant. Some of the root genes herein were discovered and characterized from

a much larger set of genes in experiments designed to find genes that cause phenotypic changes in root morphology. Such morphological changes include primary and lateral root number, size and length, as well as phenotypic changes of other parts of that plant associated with changes in root morphology.

5 In these experiments, root genes were identified by either (1) ectopic expression of a cDNA in a plant or (2) mutagenesis of the plant genome. The plants were then cultivated under standardized conditions and any phenotypic differences recorded between the modified plants as compared with the parent plant. The gene(s) causing the changes were deduced from the cDNA inserted or disrupted gene. Phenotypic differences were observed in:

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of Arabidopsis plants and compared to mRNA isolated from the aerial portion of the plants utilizing microarray procedures. The MA\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108594, 108433, 108599, 108434, 108439 ). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Roots genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff tables with a "+" or "-" indication or that are shown as having differential expression in the AFLP\_diff table.

#### Roots Genes Identified By Cluster Analyses Of Differential Expression

##### Roots Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Roots genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108594, 108433, 108599, 108434, 108439 of the MA\_diff and/or AFLP\_diff table(s).

##### Roots Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Roots genes. A group in the MA\_clust is considered a Roots pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

#### Roots Genes Identified By Amino Acid Sequence Similarity

Roots genes from other plant species typically encode polypeptides that share amino acid



similarity to the sequences encoded by corn and Arabidopsis Roots genes. Groups of Roots genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Roots pathway or network is a group of proteins that also exhibits Roots functions/utilities.

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Examples of phenotypes, biochemical activities, and transcription profiles that can be modulated by these genes and gene products are described above and below.

b. Identification of Root Genes

b. Use of Root Genes to Modulate Phenotypes

10 The root genes of the instant invention are capable of modulating one or more processes of root structure and/or function including (I) development; (II) interaction with the soil and soil contents; and (III) transport in the plant.

Root genes and gene products can be used to alter or modulate one or more of the following phenotypes.

I. DEVELOPMENT

Roots arise from meristem cells that are protected by a root cap during root elongation, but as the root grows out, the cap cells abscise and the remaining cells differentiate to the tip. Depending on the plant species, some surface cells of roots can develop into root hairs. Some roots persist for the life of the plant; others gradually shorten as the ends slowly die back; some may cease to function due to external influences. The root genes and gene products of this invention are useful to modulate any one or all of these growth and development processes generally, as in root density and root growth; including rate, timing, direction, size, for example.

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A. Different Types Of Roots

Root genes and gene products are useful to modulate either the growth and development or other processes in one or more of the following types of roots:

1. Primary
2. Lateral
3. Root Hairs (See The Section Below For More Detail)
4. Adventitious

B. Cell Properties

Root genes and gene products are useful to modulate cellular changes in:

1. Cell Size
2. Cell Division, Rate Direction And/Or Number
3. Cell Elongation
4. Cell Differentiation
5. Lignified Cell Walls
6. Epidermal Cells, Such As Trichoblasts
7. Root Apical Meristem Cells (Growth And Initiation)

C. Root Architecture

The following parts of roots can be modulated by these genes root and gene products to affect root architecture:

1. Epidermis
2. Cortex
  - (a) Epidermis
  - (b) Hypodermis
  - (c) Endodermis
  - (d) Casparian Strips
  - (e) Suberized Secondary Walls
  - (f) Parenchyma
  - (g) Aerenchyma
3. Stele
  - (a) Vacuature

- (i) Xylem
- (ii) Phloem
- (b) Pericycle

- 4. Vasculature
- 5. Xylem
- 6. Phloem
- 7. Root Cap
- 8. Root Apical Meristem
- 9. Elongating Region
- 10. Symmetry

D. Root Responses

The polynucleotides and polypeptides of this invention can be used to control the responses to internal plant programs as well as to environmental stimuli in:

- 1. Seminal System
- 2. Nodal System
- 3. Hormone Systems
  - (a) Auxin (For More Details See The Section On Auxin-Responsive Genes)
  - (b) Cytokinin (Inhibitory For Root Development, See Section On Cytokinin-Responsive Genes)
- 4. Root Cap Abscission
- 5. Root Senescence
- 6. Gravitropism
- 7. Coordination Of Root Growth And Development With That Of Other Organs, Examples Include:
  - (a) Leaves
  - (b) Flowers
  - (c) Seeds
  - (d) Fruits
  - (e) Stems
- 8. Changes In Soil Environment (For More Detail See Below)

- (a) Water
- (b) Minerals
- (c) Ph
- (d) Microfauna And Flora

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## II. INTERACTION WITH SOIL AND SOIL CONTENTS

Roots are sites of intense chemical and biological activities and as a result can strongly modify the soil they contact. Roots coat themselves with surfactants and mucilage to facilitate these activities. Specifically, roots are responsible for nutrient uptake by mobilizing and assimilating water, organic and inorganic compounds, ions and attracting and interacting with beneficial microfauna and flora. Roots also help to mitigate the effects of toxic chemicals, pathogens and stress. Examples of root properties and activities that the genes and gene products of this invention are useful to modulate are as follows:

### A. Root Surfactants And Mucilage

- 1. Mucilage
  - (a) Composition
  - (b) Secretion Rate And Time
- 2. Surfactant

### B. Nutrient Uptake Of

- 1. Water
  - (a) Which Can Be Measured By The Supply To Shoot On The Basis Of Volume/Dry Weight Or Surface Area
- 2. Nitrate And Other Sources Of Nitrogen (For Detail See The Section On Nitrogen Responsive Genes)
- 3. Phosphate
- 4. Potassium
- 5. Micronutrients (E.G. Iron, Copper, Etc.)

### C. Microbes And Nematodes Associations:

- 1. Bacteria Including Nitrogen-Fixing Bacteria
- 2. Mycorrhizae
- 3. Nodule-Forming And Other Nematodes

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- D. Oxygen
1. Transpiration
- E. Detoxification Of
1. Iron
2. Aluminum
3. Cadmium
4. Mercury
5. Salt
6. Other Heavy Metals And Toxins
- F. Pathogen Interactions/Control
1. Chemical Repellents
- (a) Glucosinolates (GSL), Which Release Pathogen-Controlling Isothiocyanates
- G. Changes In Soil Properties, Such As:
1. Ph
2. Mineral Depletion
3. Rhizosheath

### III. TRANSPORT OF MATERIALS IN PLANTS

Uptake of nutrients by roots produces a "source-sink" effect in a plant. The greater the source of nutrients, the larger "sinks," such as stems, leaves, flowers, seeds, fruits, etc. can grow. Thus, root genes and gene products are useful to modulate the vigor and yield of the plant overall as well as distinct cells, organs, or tissues. Examples are as follows:

- A. Vigor
1. Plant Nutrition
2. Growth Rate
- Whole Plant, Including Height, Flowering Time, Etc.
  - Seedling
  - Coleoptile Elongation
  - Young Leaves

- Stems
- Flowers
- Seeds
- Fruit

3. Yield

(A) Biomass

- Fresh And Dry Weight During Any Time In Plant Life, Including Maturation And Senescence

(b) Root/Tuber Yield

- Number, Size, Weight, Harvest Index
- Content And Composition, E.G. Amino Acid, Jasmonate, Oil, Protein And Starch

(c) Number Of Flowers

(d) Seed Yield

- Number, Size, Weight, Harvest Index
- Content And Composition, E.G. Amino Acid, Jasmonate, Oil, Protein And Starch

(e) Fruit Yield

- Number, Size, Weight, Harvest Index, Post Harvest Quality
- Content And Composition, E.G. Amino Acid, Jasmonate, Oil, Protein And Starch

Additional Uses Of Plants With Modified Roots

Plants with roots modified in one or more of the properties described above in I, II or III are used to provide:

- A. Higher vigor and yield of plants and harvested products due to pathogen resistance from conditioning the soil with plant-derived chemicals and/or more tolerance to stresses such as drought, flooding and anoxia.
- B. Better Animal (Including Human) Nutrition
- C. Improved Dietary Mineral Nutrition

- D. Better Plant Survival
  - (a) Decreased Lodging
  - (b) More Efficient Transport
  - (c) More Efficient Physiology
  - (d) More Efficient Metabolism
- E. Better Resistance To Plant Density Effects
- F. Increased Yield Of Valuable Molecules
- G. More Efficient Root Nodulation
- H. Better Access To Rhizobia Spray Application, For Anaerobic Soils
- I. Easier Crop Harvesting And Ground Tillage
- J. Decreased Soil Erosion

To regulate any of the phenotype(s) above, activities of one or more of the root genes or gene products is modulated and tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266) and/or screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and visually inspected for the desired phenotype or metabolically and/or functionally assayed according to Dolan et al. (1993, Development 119: 71-84), Dolan et al. (1997, Development 124: 1789-98), Crawford and Glass (1998, Trends Plant Science 3: 389-95), Wang et al. (1998, PNAS USA 95: 15134-39), Gaxiola et al. (1998, PNAS USA 95: 4046-50), Apse et al. (1999, Science 285: 1256-58), Fisher and Long (1992, Nature 357: 655-60), Schneider et al. (1998, Genes Devel 12: 2013-21) and Hirsch (1999, Curr Opin Plant Biol. 2: 320-326).

c. Use of Root Genes to Modulate Phenotypes

c. Use of Root Genes to Modulate Biochemical Activities

The activities of one or more of the root genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Association Of Root Morphology With Nitrogen Fixing Bacteria	<ul style="list-style-type: none"> <li>• Cell-Cell Recognition</li> <li>• Cell Wall Degradation</li> </ul>	Gage et al. (1996) J Bacteriol 178: 7159-66
Primary Root, Lateral Root, And Root Hair <ul style="list-style-type: none"> <li>• Initiation</li> <li>• Spacing</li> <li>• Elongation</li> <li>• Branching</li> </ul>	<ul style="list-style-type: none"> <li>• Cell Division/Elongation</li> <li>• Cell Differentiation</li> <li>• Cell Expansion</li> <li>• Auxin Mediated Response Pathways</li> </ul>	Schneider et al. (1998) Genes Devel 12: 2013-21 Casimiro et al. (2001). Plant Cell 13:843-852. Rogg et al. (2001). Plant Cell 13:465-480. Gaedeke et al. (2001). EMBO J. 20:1875-1887. Neuteboom et al. (1999). Plant Mol. Biol. 39:273-287. Schindelman et al. (2001). Genes and Dev. 15:1115- 1127. Rashotte et al. (2001) Plant Cell 13:1683-1697. Zhang et al. (2000). J Exp Bot 51:51-59. Zhang et al. (1998) Science 279: 407-409.
Metabolism	<ul style="list-style-type: none"> <li>• Organic Molecule Export</li> </ul>	Moody et al. (1988) Phytochemistry 27: 2857-61.
	<ul style="list-style-type: none"> <li>• Ion Export</li> </ul>	Uozumi et al. (2000) Plant Physiol 122: 1249-59 Frachisse et al. (2000) Plant J 21: 361-71



PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	<ul style="list-style-type: none"> <li>Nutrient Uptake</li> </ul>	<p>Frachisse et al. (2000) Plant J 21: 361-71</p> <p>Uozumio et al. (2000) Plant Physiol 122: 1249-59</p> <p>Williamson et al. (2001). Plant Physiol. 126:875-882.</p> <p>Zhang et al. (2000). J Exp Bot 51:51-59.</p> <p>Zhang et al. (1998). Science 279:4 07-409.</p> <p>Coruzzi et al. (2001). Plant Physiol. 125: 61-64.</p>
Root Gravitropism And Waving	<ul style="list-style-type: none"> <li>Reactive Oxygen Species (ROS) Such As Superoxide Anions And H<sub>2</sub>O<sub>2</sub> Production</li> <li>Auxin Transport Pathways</li> <li>Flavonoid Inhibition Of Auxin Transport Function</li> <li>Changes In Root Cap Ph</li> <li>Starch Synthesis And Storage</li> <li>Cell Differentiation</li> <li>Cell Elongation</li> </ul>	<p>Joo et al. (2001) Plant Physiol. 126:1055-60.</p> <p>Vitha et al. (2000). Plant Physiol. 122: 453-461.</p> <p>Tasaka et al. (2001) Int Rev Cytol 206:135-54.</p> <p>Brown et al. (2001) Plant Physiol 126:524-35.</p> <p>Fasano et al. (2001) Plant Cell 13:907-22.</p> <p>MacCleery et al. (1999). Plant Physiol 120:183-92</p> <p>Blancaflor et al. (1998). Plant Physiol 116:213-22</p> <p>Schneider et al. (1998) Genes Devel 12: 2013-21</p>

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Other biological activities that can be modulated by the root genes and gene products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table.

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A.1.a. USE OF ROOT GENES TO MODULATE TRANSCRIPTION  
LEVELS OF PLANT GENES

Many genes are “up regulated “ or “down regulated” because they belong to networks or cascades of genes. Thus some root genes are capable of regulating many other gene activities via these networks and hence complex phenotypes. Examples of transcription profiles of root genes are described in the Table below with associated biological activities. “Up-regulated” profiles are those where the concentrations of the mRNA in total mRNA are higher in roots as compared to aerial parts of a plant; and vice-versa for “down-regulated” profiles.

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TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
Up Regulated Transcripts	<p>Genes Expressed In Root Development</p> <p>Responders To Micro-Organismal Symbionts And Parasites</p> <p>Genes involved in polar auxin transport</p> <p>Genes involved in starch deposition in the roots</p> <p>Genes involved in production of reactive oxygen species</p> <p>Genes involved in</p>	<ul style="list-style-type: none"> <li>• Primary Root, Lateral Root, and/or Root Hair Growth and Differentiation</li> <li>• Microorganism Perception</li> <li>• Entrapment Of Microorganismal Symbionts</li> <li>• Nutrient Uptake</li> <li>• Synthesis Of Metabolites And/Or Proteins</li> <li>• Modulation Of Transduction Pathways</li> <li>• Specific Gene Transcription Initiation</li> <li>• Nutrient Uptake Enhancement</li> <li>• Gravitropic growth of roots</li> <li>• Associations with rhizobia are stimulated</li> </ul>	<ul style="list-style-type: none"> <li>• Transporters</li> <li>• Metabolic Enzymes</li> <li>• Change In Cell Membrane Structure And Potential</li> <li>• Kinases, Phosphatases, G-Proteins</li> <li>• Transcription Activators</li> <li>• Change In Chromatin Structure And/Or Localized DNA Topology</li> <li>• Cell Wall Proteins</li> <li>• Ca<sup>++</sup> Fluctuation</li> <li>• Reactive Oxygen Species (ROS) production</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
	flavonoid synthesis		
Down-Regulated Transcripts	<p>Genes Repressed In Root Development</p> <p>Responders To Micro-Organismal Symbionts And Parasites</p> <p>Genes With Discontinued Expression Or Unstable mRNA In Presence Of Root And/Or Micro-Organismal Symbionts</p>	<ul style="list-style-type: none"> <li>• Negative Regulation Of Primary Root, Lateral Root, and/or Root Hair Production Released</li> <li>• Changes In Pathways And Processes Operating In Cells</li> <li>• Changes In Metabolism</li> <li>• Inhibition of root gravitropism</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> <li>• Kinases, Phosphatases, G-Proteins</li> <li>• Change In Chromatin Structure And/Or DNA Topology</li> <li>• Stability Of Factors For Protein Synthesis And Degradation</li> <li>• Metabolic Enzymes</li> </ul>

Changes in the function or development of roots are the result of modulation of the activities of one or more of these many root genes and gene products. These genes and/or products are responsible for effects on traits such as plant vigor and seed yield, especially when plants are growing in the presence of soil borne biotic or abiotic stresses or when they are growing in barren conditions or in soils depleted of certain minerals.

Root genes, gene components and gene products can act alone or in combination as described in the introduction. Of particular interest are combinations of these genes and gene products with those that modulate stress tolerance and/or metabolism. Stress tolerance and metabolism genes and gene products are described in more detail in the sections below.

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#### Use of Promoters of Root Genes

Promoters of root genes, as described in the Reference tables, for example, can be used to modulate transcription that is induced by root development or any of the root biological processes or activities above. For example, when a selected polynucleotide sequence is operably linked to a promoter of a root gene, then the selected sequence is transcribed in the same or similar temporal, development or environmentally-specific patterns as the root gene from which the promoter was taken. The root promoters can also be used to activate antisense copies of any coding sequence to achieve down regulation of its protein product in roots. They can also be used to activate sense copies of mRNAs by RNA interference or sense suppression in roots.

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I.A.2. ROOT HAIR GENES, GENE COMPONENTS AND PRODUCTS

Root hairs are specialized outgrowths of single epidermal cells termed trichoblasts. In many and perhaps all species of plants, the trichoblasts are regularly arranged around the perimeter of the root. In Arabidopsis, for example, trichoblasts tend to alternate with non-hair cells or atrichoblasts. This spatial patterning of the root epidermis is under genetic control, and a variety of mutants have been isolated in which this spacing is altered or in which root hairs are completely absent.

a) IDENTIFICATION OF ROOT HAIR GENES

Root hair genes identified herein are defined as genes, gene components and products capable of modulating one or more processes in or the function of root hairs as described below. Root hairs are capable of controlling or influencing many plant traits, also as shown below. Examples of such root hair development genes and gene products are shown in the Reference and Sequence Tables. The protein products of many of these genes are also identified in these Tables.

Root Hair Genes Identified by Differential Expression

These genes were discovered and characterized from a much larger set of genes by experiments designed to find genes whose mRNA products are associated specifically with root hairs. These experiments made use of the arabidopsis mutant "root hairless" (rhl), which does not develop root hairs. By comparing gene expression profiles of rhl roots with those of wild type roots grown in identical conditions, genes specifically expressed in root hairs were revealed. The MA\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108594, 108433). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Root Hairs genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff tables with a "+" or "-" indication

Root Hairs Genes Identified By Cluster Analyses Of Differential Expression

Root Hairs Genes Identified By Correlation To Genes That Are  
Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Root Hairs genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108594, 108433 the MA\_diff and/or AFLP\_diff table(s).

Root Hairs Genes Identified By Correlation To Genes That Cause  
Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Root Hairs genes. A group in the MA\_clust is considered a Root Hairs pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

Root Hairs Genes Identified By Amino Acid Sequence Similarity

Root Hairs genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Root Hairs genes. Groups of Root Hairs genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Root Hairs pathway or network is a group of proteins that also exhibits Root Hairs functions/utilities.

Examples of phenotypes, biochemical activities, and transcript profiles that can be modulated by these genes and gene products are described above and below.

b) USE OF ROOT HAIR DEVELOPMENT GENES TO  
MODULATE PHENOTYPES

The root hair development genes of the instant invention are useful to modulate one or more processes of root hair structure and/or function including (I) development; (II) interaction

with the soil and soil contents; (III) uptake and transport in the plant; and (IV) interaction with microorganisms.

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## I. DEVELOPMENT

10 The surface cells of roots can develop into single epidermal cells termed trichoblasts or root hairs. Some of the root hairs will persist for the life of the plant; others will gradually die back; some may cease to function due to external influences. The genes and gene products of this invention are useful to modulate any one or all of these growth and development process generally, as in root hair density or root hair growth; including rate, timing, direction, and size, for example. A more detailed list of processes that are regulated by these genes and gene products is as follows:

### A. Cell Properties

Root hair development genes and gene products modulate cellular changes in:

1. Cell Size
2. Cell Division, Rate And Direction And Number
3. Cell Elongation
4. Cell Differentiation
5. Lignified Cell Walls
6. Epidermal Cells, Such As Trichoblasts
7. Root Apical Meristem Cells (Growth And Initiation)

### B. Root Hair Architecture

The following parts of a root hair can be modulated to affect root hair architecture:

- C. Leaf Cells Under The Trichome
- D. Cells Forming The Base Of The Trichome
- E. Trichome Cells



F. Root Hair Responses

The genes and gene products of this invention are useful to modulate any one or all of the following growth and development processes in response to internal plant programs or environmental stimuli in:

1. Seminal System
2. Nodal System
3. Hormone Responses
4. Auxin (For More Details See The Section On Auxin-Responsive Genes)
5. Root Cap Abscission
6. Root Senescence
7. Gravitropism
8. Coordination Of Root Growth And Development With That Of Other Organs, Examples Include:
  - (a) Leaves
  - (b) Flowers
  - (c) Seeds
  - (d) Fruits
  - (e) Stems
9. Changes In Soil Environment (For More Detail See Below)
  - (a) Water
  - (b) Minerals
  - (c) Ph
  - (d) Microfauna And Flora

II. INTERACTION WITH SOIL AND SOIL CONTENTS

Root hairs are sites of intense chemical and biological activity and as a result can strongly modify the soil they contact. Roots hairs can be coated with surfactants and mucilage to facilitate these activities. Specifically, roots hairs are responsible for nutrient uptake by mobilizing and assimilating water, reluctant ions, organic and inorganic compounds and

chemicals. In addition, they attract and interact with beneficial microfauna and flora. Root hairs also help to mitigate the effects of toxic ions, pathogens and stress. Examples of root hair properties and activities that the genes and gene products of the invention are useful to modulate are as follows:

- 5           A.     Root Hair Surfactant And Mucilage
  - 1.     Mucilage
    - (a)    Composition
    - (b)    Secretion Rate And Time
  - 2.    Surfactant
- 10          B.     Nutrient Uptake
  - 1.     Water
  - 2.     Nitrate And Other Sources Of Nitrogen(For Detail See The Section  
          On Nitrogen Responsive Genes)
  - 3.     Phosphate
  - 4.     Potassium
  - 5.     Micronutrients (E.G. Iron, Copper, Etc.)
- 15          C.     Microbe And Nematode Associations
  - 1.     Bacteria Including Nitrogen-Fixing Bacteria
  - 2.     Mycorrhizae
  - 20     3.     Nodule-Forming And Other Nematodes
  - 4.     Nitrogen Fixation
- D.     Oxygen
  - 1.     Transpiration
- E.     Detoxification Effects Of
  - 25     7.     Iron
  - 8.     Aluminum
  - 9.     Cadium
  - 10.    Mercury
  - 11.    Salt
  - 30     12.    Other Soil Constituents
- F.     Pathogens

1. Chemical Repellent
  - (a) Glucosinolates (GSL), Which Release Pathogen-Controlling Isothiocyanates

G. Changes In Soil

1. Ph
2. Mineral Excess And Depletion
3. Rhizosheath

III. TRANSPORT OF MATERIALS IN PLANTS

Introduction: Uptake of the nutrients by the root and root hairs contributes a source-sink effect in a plant. The greater source of nutrients, the more sinks, such as stems, leaves, flowers, seeds, fruits, etc. can draw sustenance to grow. Thus, root hair development genes and gene products are useful to modulate the vigor and yield of the plant overall as well as of distinct cells, organs, or tissues of a plant. Examples are as follows:

A. Vigor

1. Plant Nutrition
2. Growth Rate
  - (a) Whole Plant, Including Height, Flowering Time, Etc.
  - (b) Seedling
  - (c) Coleoptile Elongation
  - (d) Young Leaves
  - (e) Stems
  - (f) Flowers
  - (g) Seeds
  - (h) Fruit
3. Yield
  - (a) Biomass
    - Fresh And Dry Weight During Any Time In Plant Life, Including Maturation And Senescence
  - (b) Number Of Flowers
  - (c) Number Of Seeds

- (d) Seed Yield
- (e) Number, Size, Weight, Harvest Index
  - Content And Composition, E.G. Amino Acid, Jasmonate, Oil, Protein And Starch
- (f) Fruit Yield
  - Number, Size, Weight, Harvest Index, Post Harvest Quality

Additional Uses of Plants with Modified Root Hairs

Plants with root hairs modified in one or more of the properties described above in I, II or III are used to provide:

- A. Higher vigor and yield of plant and harvested products due to pathogen resistance from conditioning the soil with plant-derived chemicals and/or more tolerance to stresses such as drought, flooding and anoxia
- B. Better Animal (Including Human) Nutrition
- C. Improved Dietary Mineral Nutrition
- D. Increased Plant Survival By Decreasing Lodging
- E. Better Plant Survival By:
  - (a) Decreased Lodging
  - (b) More Efficient Transport
  - (c) More Efficient Physiology
  - (d) More Efficient Metabolism
- F. Increased Yield Of Valuable Molecules

Root Hair Modulation

To regulate any of the phenotype(s) above, activities of one or more of the root hair genes or gene products is modulated and tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels are altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266) and/or screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and visually inspected for the desired

phenotype or metabolically and/or functionally assayed according to Dolan et al. (1993, Development 119: 71-84), Dolan et al. (1997, Development 124: 1789-98), Crawford and Glass (1998, Trends Plant Science 3: 389-95), Wang et al. (1998, PNAS USA 95: 15134-39), Gaxiola et al. (1998, PNAS USA 95: 4046-50), Apse et al. (1999, Science 285: 1256-58), Fisher and  
 5 Long (1992, Nature 357: 655-60), Schneider et al. (1998, Genes Devel 12: 2013-21) and Hirsch (1999, Curr Opin Plant Biol. 2: 320-326).

A.2.a. USE OF ROOT HAIR DEVELOPMENT GENES TO  
MODULATE BIOCHEMICAL ACTIVITIES

The activities of one or more of the root hair development genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

Process	Biochemical Or Metabolic Activities And/Or Pathways	Citations Including Assays
Association Of Root Hair With Nitrogen Fixing Bacteria	<ul style="list-style-type: none"> <li>Functions Associated With Root Hair Curling And Signal Transduction</li> </ul>	Gage et al. (1996) J Bacteriol 178: 7159-66
Root Hair <ul style="list-style-type: none"> <li>Spacing</li> <li>Initiation</li> <li>Elongation</li> </ul>		Schneider et al. (1998) Genes Devel 12: 2013-21
Metabolism	<ul style="list-style-type: none"> <li>Organic Molecule Export</li> </ul>	Moody et al. (1988) Phytochemistry 27: 2857-61
	<ul style="list-style-type: none"> <li>Ion Export</li> </ul>	Uozumi et al. (2000) Plant Physiol 122: 1249-59 Frachisse et al. (2000) Plant J 21: 361-71
Nutrient Uptake	<ul style="list-style-type: none"> <li>Nutrient Uptake</li> </ul>	Frachisse et al. (2000) Plant J 21: 361-71 Uozumio et al. (2000) Plant Physiol 122: 1249-59

Other biological activities that can be modulated by the root hair genes and gene products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table.

5

A.2.a. USE OF ROOT HAIR GENES, GENE COMPONENTS AND  
PRODUCT TO MODULATE TRANSCRIPTION LEVELS

Many genes are "up regulated" or "down regulated" in root hairs or associated with root hair formation because genes are regulated in networks. Thus some root hairs genes are useful to regulate the activities of many other genes, directly or indirectly to influence complex phenotypes. Examples of transcription profiles of root genes are described in the Table below with associated biological activities. "Up regulated" profiles are those where the mRNA levels are higher when the rhl gene is inhibited as compared to when rhl gene is not inhibited; and vice-versa for "down-regulated" profiles.

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Transcript Levels	Type Of Genes	Physiological Consequences	Examples Of Biochemical Activity
Down Regulated Transcripts	<ul style="list-style-type: none"> <li>• Genes Expressed In Root Hair Development</li> <li>• Responders To Micro-Organismal Symbionts And Parasites</li> </ul>	<ul style="list-style-type: none"> <li>• Root Hair Formation</li> <li>• Microorganism Perception</li> <li>• Entrapment Of Microorganismal Symbionts</li> <li>• Nutrient Uptake</li> <li>• Synthesis Of Metabolites And/Or Proteins</li> <li>• Modulation Of Transduction Pathways</li> <li>• Specific Gene Transcription Initiation</li> <li>• Nutrient Uptake Enhancement</li> </ul>	<ul style="list-style-type: none"> <li>• Transporters</li> <li>• Metabolic Enzymes</li> <li>• Change In Cell Membrane Structure And Potential</li> <li>• Kinases, Phosphatases, G-Proteins</li> <li>• Transcription Activators</li> <li>• Change In Chromatin Structure And/Or Localized DNA Topology</li> <li>• Cell Wall Proteins</li> </ul>
Up-Regulated Transcripts	<ul style="list-style-type: none"> <li>• Genes Repressed In Roots Making Hairs</li> <li>• Responders To Micro-Organismal Symbionts And</li> </ul>	<ul style="list-style-type: none"> <li>• Negative Regulation Of Hair Production Released</li> <li>• Changes In Pathways And Processes Operating In Cells</li> <li>• Changes In</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> <li>• Kinases, Phosphatases, G-Proteins</li> <li>• Change In Chromatin Structure And/Or DNA Topology</li> <li>• Stability Of Factors</li> </ul>

Transcript Levels	Type Of Genes	Physiological Consequences	Examples Of Biochemical Activity
	Parasites  • Genes With Discontinued Expression Or UnsTable mRNAIn Presence Of Root Hairs And/Or Micro-Organismal Symbionts	Metabolism	For Protein Synthesis And Degradation • Metabolic Enzymes • Cell Wall Proteins

Changes in the patterning or development of root hairs are the result of modulation of the activities of one or more of these many root hair genes and gene products. These genes and/or products are responsible for effects on traits such as plant vigor and seed yield, especially when plants are growing in the presence of biotic or abiotic stresses or when they are growing in barren conditions or in soils depleted of certain minerals.

Root hair genes and gene products can act alone or in combination as described in the introduction. Of particular interest are combination of these genes and gene products with those that modulate stress tolerance and/or metabolism. Stress tolerance and metabolism genes and gene products are described in more detail in the sections below.

#### Use of Promoters of Root Hair Genes

Promoters of root hair development genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by root hair development or any of the following phenotypes or biological activities above. For example, any desired sequence can be



transcribed in similar temporal, tissue, or environmentally-specific patterns as the root hair genes when the desired sequence is operably linked to a promoter of a root hair responsive gene.

I.B. LEAF GENES, GENE COMPONENTS AND PRODUCTS

I.B.1. LEAF GENES, GENE COMPONENTS AND PRODUCTS

5 Leaves are responsible for producing most of the fixed carbon in a plant and are critical to plant productivity and survival. Great variability in leaf shapes and sizes is observed in nature. Leaves also exhibit varying degrees of complexity, ranging from simple to multi-compound. Leaf genes as defined here, not only modulate morphology, but also influence the shoot apical meristem, thereby affecting leaf arrangement on the shoot, internodes, nodes, axillary buds, photosynthetic capacity, carbon fixation, photorespiration and starch synthesis. Leaf genes elucidated here can be used to modify a number of traits of economic interest from leaf shape to plant yield, including stress tolerance, and to modify the efficiency of synthesis and accumulation of specific metabolites and macromolecules.

B.1.a. IDENTIFICATION OF LEAF GENE, GENE COMPONENTS AND PRODUCTS

10 Leaf genes identified herein are defined as genes, active or potentially active to greater extent in leaves than in some other organs of the plant or as genes that affect leaf properties. These genes and gene components are useful for modulating one or more processes in or functions of leaves, as described below, to improve plant traits ranging from yield to stress tolerance. Examples of such leaf genes and gene products are shown in the Reference and Sequence Tables and sequences encoding polypeptides of the Protein Group and Protein Group Matrix tables or fragments thereof, Knock-In, Knock-Out and MA\_diff and/or AFLP\_diff Tables. The biochemical functions of the protein products of many of these genes determined from comparisons with known proteins are also given in the Reference tables.

25 Leaf Genes Identified by Phenotypic Observations

Some leaf genes were discovered and characterized from a much larger set of genes by experiments designed to find genes that cause phenotypic changes in leaf, petiole, internode, and cotyledon morphology.

30 In these experiments, leaf genes were identified by either (1) ectopic expression of a cDNA in a plant or (2) mutagenesis of the plant genome. The plants were then cultivated and

one or more of the following leaf phenotypes, which varied from the parental "wild-type", were observed:

A. Changes In Seedling Stage Cotyledons

- Cup Shaped
- Curled
- Horizontally Oblong
- Long Petioles
- Short Petioles
- Silver
- Tricot
- Wilted

B. Changes In Rosette And Flowering Stage Leaf Shapes

- Cordate
- Cup-Shaped
- Curled
- Fused
- Lanceolate
- Lobed
- Long Petioles
- Short Petioles
- Oval
- Ovate
- Serrate
- Trident
- Undulate
- Vertically Oblong

C. Changes In Cauline, Flowering Leaf Shape

- Misshapen
- Other

D. Changes In Leaf Pigment

- Albino
  - Dark Green Pigment
  - High Anthocyanin
  - Interveinal Chlorosis
  - Yellow Pigment
- E. Changes In Leaf Size
- F. Changes In Seedling Stage Hypocotyl
- Long
  - Short
- G. Changes In Leaf Number
- H. Changes In Wax Deposition
- Glossy Rosette And Flowering Stage Leaves
  - Altered Wax Deposition On The Bolt

#### Leaf Genes Identified by Differential Expression

Also, leaf genes were identified in experiments in which the concentration of mRNA products in the leaf, or stem, or Knock-out mutant 3642-1 were compared with to a control. The MA\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108477, 108512, 108497, 108498, 108598). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Leaf genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### Leaf Genes Identified By Cluster Analyses Of Differential Expression

##### Leaf Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table

indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Leaf genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108477, 108512, 108497, 108498, 108598 of the MA\_diff and/or AFLP\_diff table(s).

Leaf Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Leaf genes. A group in the MA\_clust is considered a Leaf pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

Leaf Genes Identified By Amino Acid Sequence Similarity

Leaf genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Leaf genes. Groups of Leaf genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Leaf pathway or network is a group of proteins that also exhibits Leaf functions/utilities.

It is assumed that (i) the genes preferentially expressed in leaves are concerned with specifying leaf structures and the synthesis of all the constituent molecules and (ii) that the genes repressed in leaves specify products that are not required in leaves or that could inhibit normal leaf development and function.

Examples of phenotypes, biochemical activities, and transcription profiles that are modulated by using selected members of these genes and gene products, singly or in combination, are described below.

B.1.b. USE OF LEAF GENES, GENES COMPONENTS AND  
PRODUCTS TO MODULATE PHENOTYPES

Leaves are critical for the performance and industrial utility of plants. There is extensive evidence that the number, size, shape, position, timing of synthesis, timing of senescence and chemical constitution are very important for agriculture, horticulture and uses of plants as chemical factories for making valuable molecules. Many improvements already demonstrated over past decades have involved genetic modifications to leaves. Therefore, the leaf genes and gene components of this invention offer considerable opportunities for further improving plants for industrial purposes. When the leaf genes and/or gene components are mutated or regulated differently, they are capable of modulating one or more of the processes determining leaf structure and/or function including (I) development; (II) interaction with the environment and (III) photosynthesis and metabolism.

I. DEVELOPMENT

The leaf genes, gene components and products of the instant invention are useful to modulate one or more processes of the stages of leaf morphogenesis including: stage 1- organogenesis that gives rise to the leaf primordium; stage 2- delimiting basic morphological domains; and stage 3- a coordinated processes of cell division, expansion, and differentiation. Leaf genes include those genes that terminate as well as initiate leaf development. Modulating any or all of the processes leads to beneficial effects either at specific locations or throughout the plant.

A. Gene Sequences Affecting Types of Leaves

Applicants provide with these genes, gene components and gene products the means to modulate one or more of the following types of leaves, shoots, and stems:

1. Cotyledons
2. Major Leaves
3. Cauline Leaves
4. Petioles

B. Gene Sequences Affecting Cell properties

Leaf genes, gene components and gene products are useful to modulate changes in:

1. Cell Size
2. Cell Division, Rate And Direction
3. Cell Elongation
4. Cell Differentiation
5. Stomata Size, Number, Spacing And Activity
6. Trichome Size And Number (For More Details See Section On Trichome Genes)
7. Xylem And Phloem Cell Numbers
8. Cell Wall Composition
9. All Cell Types

C. Gene Sequences Affecting Leaf Architecture:

The following properties of a leaf are useful to modulate to change overall leaf architecture:

1. Veination – Improvements in photosynthetic efficiency, stress tolerance efficiency of solute and nutrient movement to and from the leaf are accomplished by increases or decreases in:
  - (a) Vein Placement
  - (b) Number Of Cells In The Vein
2. Shape
  - (a) Elongated Versus Rounded
  - (b) Symmetry, Around Either
    - Abaxial-Adaxial (Dorsiventral) Axis
    - Apical-Basal (Proximodistal) Axis
    - Margin-Blade-Midrib (Lateral) Axis
3. Branching – Improved plant performance to biotic and abiotic stress in heavy density planting is achieved by increases or decreases in:
  - (a) Leaf branch position
  - (b) Leaf branch length

G. Genes Sequences Influencing Leaf Responses

Shoot apical meristem cells differentiate to become leaf primordia that eventually develop into leaves. The genes, gene components and gene products of this invention are useful to modulate any one or all of these growth and development processes, by affecting timing and rate or planes of cell divisions for example, in response to the internal plant stimuli and/or programs listed below:

1. Embryogenesis
2. Germination
3. Hormones
  - (a) Auxin (For More Details See The Section On Auxin-Responsive Genes)
4. Leaf Senescence
5. Phototropism
6. Coordination Of Leaf Growth And Development With That Of Other Organs
  - (a) Roots
  - (b) Flowers
  - (c) Seeds
  - (d) Fruits
  - (e) Stems
7. Stress-Related Programs

II. INTERACTION WITH THE ENVIRONMENT

Leaves are the main sites of photosynthesis and have various adaptations for that purpose. Flat laminae provide a large surface for absorbing sunlight; leaves are rich in chloroplasts and mitochondria; stomata in the lower surface of the laminae allow gases to pass into and out of the leaves including water; and an extensive network of veins brings water and minerals into the leaves and transports the sugar products produced by photosynthesis to the rest of the plant. Examples of leaf properties or activities that are modulated by leaf genes, gene components and their products to facilitate interactions between a plant and the environment include:



- 5
- 10
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- 20
- A. Pigment Accumulation (See The Section On Viability Genes For More Detail)
  - B. Wax accumulation on the surface of leaves – Improved protection of young leaves from water borne pathogen attack such as Downey Mildew with increased wax production.
  - C. Oxygen Gain/Loss Control
  - D. Carbon Dioxide Gain/Loss Control
  - E. Water Gain/Loss Control
  - F. Nutrient Transport
  - G. Light Harvesting
  - H. Chloroplast Biogenesis
  - I. Circadian Rhythm Control
  - J. Light/Dark Adaptation
  - K. Defense Systems Against Biotic And Abiotic Stresses
  - L. Metabolite Accumulation
  - M. Secondary metabolite production in leaf mesophyl, epidermis and trichomes.
    - (a) Increases in antifeeding secondary metabolites such as strictosiden reduce herbivory.
    - (b) Decreases in secondary metabolites improve plants as forage by reducing allergens or undigestible compounds.

### III. PHOTOSYNTHESIS AND METABOLISM

25 Many of the uses for plants depend on the success of leaves as the powerhouses for plant growth, their ability to withstand stresses and their chemical composition. Leaves are organs with many different cell types and structures. Most genes of a plant are active in leaves and therefore leaves have very diverse of pathways and physiological processes. Examples of such pathways and processes that are modulated by leaf genes, gene components and products include:

- 30
- A. Photosynthesis
  - B. Sugar Metabolism

- C. Starch Synthesis
- D. Starch Degradation
- E. Nitrate And Ammonia Metabolism
- F. Amino Acid Biosynthesis, Transport
- G. Protein Biosynthesis
- H. DNA Replication, Repair
- I. Lipid Biosynthesis And Breakdown
- J. Protein Biosynthesis, Storage And Breakdown
- K. Nucleotide Transport And Metabolism
- L. Cell Envelope Biogenesis
- M. Membrane Formation
- N. Mitochondrial And Chloroplast Biogenesis
- O. Transcription And RNA Metabolism
- P. Vitamin Biosynthesis
- Q. Steroid And Terpenoid Biosynthesis
- R. Devise Secondary Metabolite Synthesis
- S. Co-Enzyme Metabolism
- T. Flavonoid Biosynthesis And Degradation
- U. Synthesis Of Waxes
- V. Glyoxylate Metabolism
- W. Hormone Perception And Response Pathways

Uses of Plants that Are Modified as Described Above

Altering leaf genes or gene products in a plant modifies one or more of the following plant traits, to make the plants more useful for specific purposes in agriculture, horticulture and for the production of valuable molecules. The useful plants have at least one of the following:

- A. A higher yield of leaves and their molecular constituents due to different:
  - 1. Number, Size, Weight, Harvest Index
  - 2. Composition Including And Amounts And Types Of Carbohydrates, Proteins, Oils, Waxes, Etc.

3.     Photosynthetic Efficiency E.G. Reduced Photorespiration
  4.     Absorption Of Water And Nutrients To Enhance Yields,  
         Including Under Stresses Such As High Light, Herbicides,  
         And Heat.
  5.     Pathways To Accumulate New Valuable Molecules.
- B.    More optimal leaf shape and architecture – enhancing photosynthesis and  
      enhancing appeal in ornamental species
- (a)    Size
  - (b)    Number
  - (c)    Pigment
  - (d)    Aroma
- C.    A better overall plant architecture – enhancing photosynthesis and  
      enhancing appeal in ornamental species
- (a)    Petals
  - (b)    Sepals
  - (c)    Stamens
  - (d)    Carpels
- D.    Better shade avoidance for maximizing photosynthesis by, for example,  
      altering leaf placement, to improve light capture and photosynthetic  
      efficiency, thereby increasing yields
- E.    Reduced negative effects of high planting density, by altering leaf  
      placement to be more vertical instead of parallel to the ground, for  
      instance
- F.    More resistance to the deleterious effects of wind and mechanical damage.
- G.    Better stress tolerance, including without limitation
1.     Drought resistance, by decreasing water loss, for example
  2.     Pathogen resistance, including, for instance,  
          Insect resistance through internal insecticide levels and  
          optimizing the leaf shape to prevent runoff of insecticides
- H.    Better overall yield and vigor

Plant yield of biomass and of constituent molecules and plant vigor are modulated to create benefits by genetically changing:

1. Growth Rate Of
  - (a) Whole Plant, Including Height, Flowering Time, Etc.
  - (b) Seedling
  - (c) Coleoptile Elongation
  - (d) Young Leaves
  - (e) Flowers
  - (f) Seeds
  - (g) Fruit
2. Biomass
  - (a) Fresh And Dry Weight During Any Time In Plant Life, Including Maturation And Senescence
  - (b) Number Of Flowers
  - (c) Seed Yield Including, For Example,
    - Number, Size, Weight, Harvest Index
    - Content And Composition, E.G. Amino Acid, Jasmonate, Oil, Protein And Starch
  - (d) Fruit Yield
    - Number, Size, Weight, Harvest Index
    - Content And Composition, E.G. Amino Acid, Jasmonate, Oil, Protein And Starch

To change any of the phenotype(s) in I, II, or III above, activities of one or more of the leaf genes or gene products are modulated in an organism and the consequence evaluated by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels are altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (Methods. Mol. Biol. 82:259-266 (1998)) with leaf gene constructs and/or screened for variants as in Winkler et al., Plant Physiol. 118: 743-50 (1998) and visually inspected for the desired phenotype and metabolically and/or functionally assayed for altered levels of relevant molecules.

B.1.c. USE OF LEAF GENES, GENE COMPONENTS AND PRODUCTS TO  
MODULATE BIOCHEMICAL ACTIVITIES

Leaves are complex organs and their structure, function and properties result from the integration of many processes and biochemical activities. Some of these are known from the published literature and some can be deduced from the genes and their products described in this application. Leaf genes, and gene components are used singly or in combination to modify these processes and biochemical activities and hence modify the phenotypic and trait characteristics described above. Examples of the processes and metabolic activities are given in the Table below. The resulting changes are measured according to the citations included in the Table.

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Metabolism – anabolic and catabolic	<ul style="list-style-type: none"> <li>• Farnesylation</li> <li>• Cell Wall Biosynthesis</li> <li>• Nitrogen Metabolism</li> <li>• Secondary Metabolite Biosynthesis and Degradation</li> </ul>	Pei et al., <u>Science</u> <u>282</u> : 287-290 (1998); Cutler et al., <u>Science</u> <u>273</u> : 1239 (1996) Goupil et al., <u>J Exptl. Botany</u> <u>49</u> :1855-62 (1998) Walch-Liu et al., <u>J Exppt. Botany</u> <u>51</u> , 227-237 (2000)
Water Conservation And Resistance To Drought And Other Related Stresses	<ul style="list-style-type: none"> <li>• Stomatal Development And Physiology</li> <li>• Production of polyols</li> <li>• Regulation of salt concentration</li> <li>• ABA response(s)</li> <li>• Ca<sup>2+</sup> Accumulation</li> <li>• K<sup>+</sup> Fluxes</li> </ul>	Allen et al., <u>Plant Cell</u> <u>11</u> : 1785-1798 (1999) Li et al., <u>Science</u> <u>287</u> : 300-303 (2000) Burnett et al., <u>J Exptl. Botany</u> <u>51</u> : 197-205 (2000) Raschke, In: <u>Stomatal Function</u> , Zeiger et al. Eds., 253-279 (1987)

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Transport Anion and Cation Fluxes	<ul style="list-style-type: none"> <li>• Na<sup>+</sup> Fluxes</li> <li>• Receptor – ligand binding</li> <li>• Anion and Cation fluxes</li> </ul>	<p>Lacombe et al., <u>Plant Cell</u> <b>12</b>: 837-51 (2000);</p> <p>Wang et al., <u>Plant Physiol.</u> <b>118</b>:1421-1429 (1998);</p> <p>Shi et al., <u>Plant Cell</u> <b>11</b>: 2393- 2406 (1999)</p> <p>Gaymard et al., <u>Cell</u> <b>94</b>:647- 655 (1998)</p> <p>Jonak et al., <u>Proc. Natl. Acad.</u> <u>Sci.</u> <b>93</b>: 11274-79 (1996);</p> <p>Sheen, <u>Proc. Natl. Acad. Sci.</u> <b>95</b>: 975-80 (1998);</p> <p>Allen et al., <u>Plant Cell</u> <b>11</b>: 1785-98 (1999)</p>
Carbon Fixation	<ul style="list-style-type: none"> <li>• Calvin Cycle <ul style="list-style-type: none"> <li>- Photorespiration</li> <li>- Oxygen evolution</li> <li>- RuBisCO</li> </ul> </li> <li>• Chlorophyll metabolism</li> <li>• Chloroplast Biogenesis and Metabolism</li> <li>• Fatty Acid and Lipid Biosynthesis</li> <li>• Glyoxylate metabolism</li> <li>• Sugar Transport</li> <li>• Starch Biosynthesis and</li> </ul>	<p>Wingler et al., <u>Philo Trans R</u> <u>Soe Lond B Biol Sci</u> <b>355</b>, 1517-1529 (2000);</p> <p>Palecanda et al., <u>Plant Mol</u> <u>Biol</u> <b>46</b>, 89-97 (2001);</p> <p>Baker et al., <u>J Exp Bot</u> <b>52</b>, 615-621 (2001)</p> <p>Chen et al., <u>Acta Biochim Pol</u> <b>41</b>, 447-457 (1999)</p> <p>Imlau et al., <u>PlantCell</u> <b>II</b>, 309- 322 (1999)</p>

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	Degradation	
Hormone Perception and Growth	<ul style="list-style-type: none"> <li>• Hormone Receptors and Downstream Pathways for <ul style="list-style-type: none"> <li>- ethylene</li> <li>- jasmonic acid</li> <li>- brassinosteroid</li> <li>- gibberellin</li> <li>- auxin</li> <li>- cytokinin</li> </ul> </li> <li>• Activation Of Specific Kinases And Phosphatases</li> </ul>	<p>Tieman et al., <u>Plant J</u> <u>26</u>, 47-58 (2001)</p> <p>Hilpert et al., <u>Plant J</u> <u>26</u>, 435-446 (2001)</p> <p>Wenzel et al., <u>Plant Phys</u> <u>124</u>, 813-822 (2000)</p> <p>Dengler and Kang, <u>Curr Opin Plant Biol</u> <u>4</u>, 50-56 (2001)</p> <p>Tantikanjana et al., <u>Genes Dev</u> <u>15</u>, 1577-1580 (2001)</p>

Other biological activities that are modulated by the leaf genes and gene products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table, for example.

B.1.d. USE OF LEAF GENES, GENE COMPONENTS AND PRODUCTS TO  
MODULATE TRANSCRIPTION LEVELS

The expression of many genes is “upregulated” or downregulated” in leaves because  
5 some leaf genes and their products are integrated into complex networks that regulate  
transcription of many other genes. Some leaf genes, gene components and products are therefore  
useful for modifying the transcription of other genes and hence complex phenotypes, as  
described above. Profiles of leaf gene activities are described in the Table below with associated  
biological activities. “Up-regulated” profiles are those where the mRNA transcript levels are  
10 higher in leaves as compared to the plant as a whole. “Down-regulated” profiles represent higher  
transcript levels in the whole plant as compared to leaf tissue only.



TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENE PRODUCTS WITH MODIFIED LEVELS
Up Regulated Transcripts	Genes Involved In Leaf Cell Differentiation, Cell Division, Cell Expansion	<ul style="list-style-type: none"> <li>• Leaf Cells Proliferate And Differentiate;</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors, Signal Transduction Proteins, Kinase And Phosphatases</li> </ul>
	Genes Involved In Positive Regulation Of Leaf Genes	<ul style="list-style-type: none"> <li>• Leaf Structures Form And Expand</li> </ul>	<ul style="list-style-type: none"> <li>• Chromatin Remodeling</li> <li>• Hormone Biosynthesis Enzymes</li> <li>• Receptors</li> </ul>
	Repressors Of Root And Other Non Leaf Cell Types		
	Genes Involved In Photosynthesis	<ul style="list-style-type: none"> <li>• Photosynthesis And Plastid Differentiation</li> </ul>	<ul style="list-style-type: none"> <li>• Light Harvesting Coupled To ATP Production</li> <li>• Chlorophyll Biosynthesis</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENE PRODUCTS WITH MODIFIED LEVELS
		<ul style="list-style-type: none"> <li>• Calvin Cycle Activated</li> <li>• Chloroplast Biogenesis And Plastid Differentiation Activated</li> </ul>	<ul style="list-style-type: none"> <li>• Ribulose Biphosphate Carboxylase</li> <li>• Chloroplast Membranes Synthesis</li> <li>• Chloroplast Ribosome Biogenesis</li> </ul>
	Other Genes Involved In Metabolism	<ul style="list-style-type: none"> <li>• Starch Biosynthesis</li> <li>• Lipid Biosynthesis</li> <li>• Nitrogen Metabolism – NO<sub>3</sub> Reduced And Amino Acids Made</li> <li>• Secondary Metabolites Produced</li> </ul>	<ul style="list-style-type: none"> <li>• Starch Synthase</li> <li>• Nitrate Reductase</li> <li>• Terpenoid Biosynthesis</li> <li>• Transcription Factors</li> <li>• Transporters</li> <li>• Kinases</li> <li>• Phosphatases And Signal Transduction Protein</li> <li>• Chromatin Structure Modulators</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENE PRODUCTS WITH MODIFIED LEVELS
Down Regulated Genes	Genes Involved In Negative Regulation Of Leaf Genes	<ul style="list-style-type: none"> <li>• Leaf Genes Activated And Leaf Functions Induced;</li> <li>• Dark-Adapted Metabolism Suppressed</li> <li>• Meristematic Genes Suppressed</li> <li>• Leaf Metabolic Pathways Induced</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> <li>• Signal Transduction Proteins – Kinases And Phosphatases</li> <li>• Metabolic Enzymes</li> <li>• Chromatin Remodeling Proteins</li> </ul>

While leaf polynucleotides and gene products are used singly, combinations of these polynucleotides are often better to optimize new growth and development patterns. Useful combinations include different leaf polynucleotides and/or gene products with a hormone responsive polynucleotide. These combinations are useful because of the interactions that exist between hormone-regulated pathways, nutritional pathways and development.

#### Use of Leaf Gene Promoters

Promoters of leaf genes are useful for transcription of desired polynucleotides, both plant and non-plant. If the leaf gene is expressed only in leaves, or specifically in certain kinds of leaf cells, the promoter is used to drive the synthesis of proteins specifically in those cells. For example, extra copies of carbohydrate transporter cDNAs operably linked to a leaf gene promoter and inserted into a plant increase the “sink” strength of leaves. Similarly, leaf

promoters are used to drive transcription of metabolic enzymes that alter the oil, starch, protein, or fiber contents of a leaf. Alternatively, leaf promoters direct expression of non-plant genes that can, for instance, confer insect resistance specifically to a leaf. Additionally the promoters are used to synthesize an antisense mRNA copy of a gene to inactivate the normal gene expression  
5 into protein. The promoters are used to drive synthesis of sense RNAs to inactivate protein production via RNA interference.

I.B.2. TRICHOME GENES AND GENE COMPONENTS

Trichomes, defined as hair-like structures that extend from the epidermis of aerial tissues, are present on the surface of most terrestrial plants. Plant trichomes display a diverse set of structures, and many plants contain several types of trichomes on a single leaf. The presence of trichomes can increase the boundary layer thickness between the epidermal tissue and the environment, and can reduce heat and water loss. In many species, trichomes are thought to protect the plant against insect or pathogen attack, either by secreting chemical components or by physically limiting insect access to or mobility on vegetative tissues. The stellate trichomes of Arabidopsis do not have a secretory anatomy, but at a functional level, they might limit herbivore access to the leaf in the field. In addition, trichomes are known to secrete economically valuable substances, such as menthol in mint plants.

B.2.a. IDENTIFICATION OF TRICHOME GENES, GENE COMPONENTS AND PRODUCTS

Trichome genes identified herein are defined as genes or gene components capable of modulating one or more processes in or functions of a trichome, as described below. These genes, their components and products are useful for modulating diverse plant traits from production of secondary metabolites to pathogen resistance. Examples of such trichome genes and gene products are shown in the Reference and Sequence Tables and sequences encoding polypeptides of the Protein Group and Protein Group Matrix tables or fragments thereof, Knock-in, Knock-out, MA-diff and MA-clust. The biochemical functions of the protein products of many of these genes determined from comparisons with known proteins are also given in the Reference tables.

Trichome Genes Identified by Phenotypic Observation

Trichome genes were discovered and characterized from a much larger set of genes by experiments designed to find genes that cause phenotypic changes in trichome number and morphology on leaf, internode, cotyledon, petiole, and inflorescence. In these experiments, trichome genes were identified by either (1) ectopic expression of a cDNA in a plant or (2) mutagenesis of the plant genome. The plants were then cultivated and one or more of the following phenotypes, which varied from parental "wild-type", were observed: (1) trichome

number; (2) trichome spacing (clustering); or (3) trichome branching. The genes regulating trichome phenotypes are identified in the Knock-In and Knock-Out Tables.

#### Trichome Genes Identified by Differential Expression

5 Trichome genes were also discovered and characterized from a much larger set of genes by experiments designed to find genes whose mRNA products are associated specifically or preferentially with trichomes. These experiments made use of an Arabidopsis glabrous mutant and a hairy mutant. By comparing gene expression profiles of the glabrous mutant with those of the hairy mutant grown under identical conditions, genes specifically or preferentially expressed in trichomes were revealed. The MA\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108452). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

10 Trichome genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### Trichome Genes Identified By Cluster Analyses Of Differential Expression

##### Trichome Genes Identified By Correlation To Genes That Are Differentially Expressed

20 As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

25 A pathway or network of Trichome genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108452 of the MA\_diff and/or AFLP\_diff table(s).

##### Trichome Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Trichome genes. A group in the MA\_clust is considered a Trichome pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section  
5 above.

#### Trichome Genes Identified By Amino Acid Sequence Similarity

Trichome genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Trichome genes. Groups of  
10 Trichome genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Trichome pathway or network is a group of proteins that also exhibits Trichome functions/utilities.

It is assumed that the genes differentially expressed in trichomes or leaves producing trichomes are concerned with specifying trichomes and their functions and therefore modulations of such genes and their products modify trichomes and their products.  
15

Examples of phenotypes, biochemical activities, and transcription profiles that can be modulated by selected numbers of these genes and gene products singly or in combinations are described above and below.

#### B.2.b. USE OF TRICHOME GENES, GENE COMPONENTS AND PRODUCTS TO MODULATE PHENOTYPES

  
20

Trichome genes of the instant invention, when mutated or activated differently, are useful for modulating one or more processes of trichome structure and/or function including: (I) development; (II) plant stress tolerance; and (III) biosynthesis or secretion of trichome-specific  
25 molecules. Trichome genes, components and gene products are useful to alter or modulate one or more of the following phenotypes:

##### I. Development

Trichome differentiation is integrated with leaf development, hormone levels and the vegetative development phase. The first trichome at the leaf tip appears only after the leaf grows  
30 to ~100  $\mu\text{m}$  in length. Subsequent events proceed basipetally as the leaf grows. As leaf development progresses, cell division patterns become less regular and islands of dividing cells

can be observed among differentiated pavement cells with their characteristic lobed morphology. Trichome initiation in the expanding leaf occurs within these islands of cells and often defines points along the perimeter of a circle, with an existing trichome defining the center.

Once a cell enters the trichome pathway it undergoes an elaborate morphogenesis  
5 program that has been divided into different stages based on specific morphological hallmarks.

Selected members of the genes, gene components and gene products of this invention are useful to modulate any one or all of these growth and development processes by affecting rate, timing, direction and size, for example. Trichome genes can also affect trichome number and the organs on which they occur. The following can be modulated by these genes:

10  
15  
A. Types of Trichomes

Applicants provide the means to modulate one or more of the following types of trichomes with the genes and gene products of this invention:

1. Glandular trichomes
2. Stellate trichomes

20  
25  
B. Cell properties

Trichome genes and gene products are useful to modulate cellular changes in:

1. Cell size
2. Cell division rate and direction
3. Cell elongation
4. Cell differentiation
5. Secretory cells
6. Trichome number (Average trichome number per leaf for mint:13,500,000).
7. Cell walls
8. Cell death
9. Response to reactive oxygen species



C. Trichome Architecture

The following parts or arrangement of trichomes on leaves can be modulated to affect trichome and/or leaf architecture:

1. Trichome cell structure
2. Placement on leaf
3. Secretory systems

D. Trichome responses

Selected members of trichome genes, gene components and gene products of this invention are useful to modulate any one or all of the growth and development processes above; as in timing and rate, for example. In addition, the polynucleotides and polypeptides of the invention can control the response of these processes to internal plant programs and signaling molecules such as:

1. Leaf development
2. Hormones:
  - (a) abscisic acid (for more details see the section on abscisic acid-responsive genes)
  - (b) auxin (for more details see the section on auxin-responsive genes)
  - (c) cytokinin (for more details see the section on cytokinin-responsive genes)
  - (d) gibberellins (for more details see the section on gibberellin-responsive genes)
  - (e) brassinosteroids (for more details see the section on brassinosteroid-responsive genes)
3. Apoptosis
4. Coordinated trichome growth and development in:
  - (a) Flowers
  - (b) Stems
  - (c) Petioles
  - (d) Cotyledons
  - (e) Hypocotyls

II. Stress Tolerance

The physical characteristics of trichomes as well as the substances secreted by trichomes are useful in protecting the plant from both biotic and abiotic attacks. Thus, selected trichome genes and gene products can be used to help protect distinct cells, organs, or tissues as well as overall plant yield and vigor. Examples of stresses, tolerances to which are modulated by trichome genes and gene products, are as follows:

- A. Drought, e.g., trichome number variation can decrease the surface area that allows evaporation
- B. Heat, e.g., trichomes can produce shade and provide protection for meristems
- C. Salt
- D. Insects, e.g., trichomes can prevent insects from settling on plant surfaces
- E. Herbivory, e.g., trichomes can produce harmful chemicals
- F. Ultraviolet light

III. Biosynthesis, Accumulation or Secretion of Metabolites

The glandular trichomes from various species are shown to secrete and, sometimes, locally synthesize a number of substances including salt, monoterpenes and sesquiterpenes, terpenoids, exudate, insect entrapping substances, antifeedants, pheromones, and others. Therefore, trichome genes can be used to modulate the synthesis, accumulation and secretion of a large number of metabolites especially related to trichome biology. Some are synthesized in response to biotic and abiotic stresses. For a more detailed description of these metabolites see the section "Use of Trichome Genes to Modulate Biochemical Activities" below.

Uses of Plants that Are Modified as Described Above

Altering trichome properties is useful for modifying one or more of the following plant traits making the plants more useful in agriculture, horticulture and for the production of valuable molecules.

- 5
- A. Production of specific carbohydrates, proteins, oils, aromas, flavors, pigments, secondary metabolites such as menthol (and other monoterpenes), etc., that can be used in situ or purified and used in a wide variety of industries.
- B. Increased production of molecules synthesized in trichomes by increasing the trichome number on different plant organs, such as cotyledons, leaves, hypocotyls, stems, petioles, etc.
- C. Increased cotton fibers per boll due to decreased numbers of trichomes that reduces insect hiding and contamination
- D. More optimal growth rate of a whole plant or specific parts of a plant due to more optimal trichome cellular development and the better resistance to biotic /abiotic stresses. Examples of specific plant parts include, without limitation,
1. Whole plant
  2. Seedling
  3. Coleoptile elongation
  4. Young leaves
  5. Flowers
  6. Seeds
  7. Fruit
- E. Increased harvested yield of plants, organs and their constituent molecules
1. Biomass
    - (a) Fresh and dry weight during any time in plant life, including maturation and senescence
    - (b) Number of flowers
    - (c) Seed yield
      - Number, size, weight, harvest index
      - Content and composition, e.g. amino acid, jasmonate, oil, protein and starch
    - (d) Fruit yield
      - Number, size, weight, harvest index, post harvest quality
      - Content and composition, e.g. amino acid, jasmonate, oil, protein and starch

To regulate any of the phenotype(s) above, activities of one or more of the trichome genes or gene products can be modulated in an organism and tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (Methods. Mol. Biol. 82:259-266 (1998)) and/or screened for variants as in Winkler et al., Plant Physiol. 118: 743-50 (1998) and visually inspected for the desired phenotype or metabolically and/or functionally assayed.

B.2.c. USE OF TRICHOME GENES, GENE COMPONENTS AND  
PRODUCTS TO MODULATE BIOCHEMICAL ACTIVITIES

The phenotype traits outlined above result from the integration of many cellular trichome associated processes and biochemical activities. Some of these are known from published literature and some can be deduced from the genes discovered in the MA Tables, etc. One or more of these trichome genes, gene components and products are useful to modulate these cellular processes, biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Growth , Differentiation And Development	<ul style="list-style-type: none"> <li>• Cell wall biosynthetic enzymes</li> <li>• Cell fate determination proteins</li> <li>• Major pathways of carbon and nitrogen metabolism</li> </ul>	<p>Molhoj et. al. (2001). Plant Mol.Biol. 46, 263-275</p> <p>Krishnakumar and Oppenheimer (1999). Development 1221, 3079-3088.</p> <p>Kroumova et al. (1994). PNAS 91, 11437-11441</p>
Water Conservation And Resistance To Drought And Other Related Stresses	<ul style="list-style-type: none"> <li>• Cytoskeleton and Trichome morphology and spacing controls</li> </ul>	<p>Schnittger et al. (1999). Plant Cell 11, 1105-1116</p> <p>Hulskamp et al (1994). Cell 76, 555-566</p>
Trichome exudate	<ul style="list-style-type: none"> <li>• Insect repellant</li> </ul>	Insects and The Plant Surface, pp 151-172,

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
		Edward Arnold, London (1986)
Terpenoid biosynthesis including monoterpenes and sesquiterpenes	<ul style="list-style-type: none"> <li>• Terpenoid biosynthesis enzymes including: <ul style="list-style-type: none"> <li>• Farnesyltranstransferase</li> <li>• Geranylgeranyl-diphosphate synthase</li> <li>• Geranyltranstransferase</li> <li>• Farnesyl-diphosphate synthase</li> <li>• Dimethylallyltranstransferase</li> <li>• Geranyl-diphosphate synthase</li> </ul> </li> </ul>	<p>Alonso et al. (1992). J. Biol. Chem. 267, 7582-7587</p> <p>Rajonarivony et al (1992). Arch. Biochem. Biophys. 299, 77-82</p>
H <sub>2</sub> O <sub>2</sub> accumulation and activation of SAR	<ul style="list-style-type: none"> <li>• NADPH oxidase (subunit) synthesis and function</li> </ul>	<p>Alvarez et al (1998) Cell 92, 773-784</p> <p>Grant Orozco-Cardenas and Ryan (1999) PNAS 96, 6553-6557</p>
Antifeedants biosynthesis and secretion	<ul style="list-style-type: none"> <li>• Lactone biosynthesis enzymes</li> </ul>	Paruch et al. (2000). J. Agric. Food Chem. 48, 4973-4977
Pheromone biosynthesis and secretion	<ul style="list-style-type: none"> <li>• Farnesine biosynthesis enzymes</li> </ul>	Teal et al. (1999) Arch. Insect Biochem Physiol. 42, 225-232
Endoreplication	<ul style="list-style-type: none"> <li>• Cyclin and cyclin dependant</li> </ul>	De Veylder et al. (2001)

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PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	kinases	Plant Cell 13, 1653-1668 De Veylder et al. (2001) Plant J. 25, 617-626

Specific enzyme and other activities associated with the functions of individual trichome genes that can be modulated by the trichome genes and gene products are listed in the Reference tables where the functions of individual genes and their products are listed. Assays for detecting such biological activities are described in the Protein Domain table, for example.

B.2.d. USE OF TRICHOME GENES, GENE COMPONENTS AND  
PRODUCTS TO MODULATE PHENOTYPES BY MODULATING  
TRANSCRIPTION LEVELS OF OTHER GENES

Many of the genes are “up regulated” or “down regulated” in trichomes because they are regulated as members of networks or cascade of genes under the control of regulatory genes. Thus some trichome genes are useful to influence levels of other genes and so orchestrate the complex phenotypes. Examples of the types of genes with altered transcript levels in trichomes are described in the Table below, together with associated biological activities. “Up-regulated” profiles are those where the mRNA levels are higher in the glabrous plants as compared to the “hairy” plant. “Down-regulated” profiles represent higher transcript levels in the “hairy” plant as compared to the glabrous plant.

TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES WHOSE TRANSCRIPTS ARE CHANGED
Up Regulated Transcripts	Genes active in suppressing trichome formation	<ul style="list-style-type: none"> <li>• Changes in Hormone Perception</li> <li>• Changes in Hormone Biosynthesis</li> <li>• Changes in Specific Gene Transcription Initiation</li> <li>• Changes in cytoskeleton and cell wall assembly and structure</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> <li>• Transporters</li> <li>• Change In Cell G-proteins</li> <li>• Kinases And Phosphatases</li> <li>• Transcription factors</li> <li>• Ca-binding proteins</li> <li>• Transcription Activators</li> <li>• Change In Chromatin Structure And/Or Localized DNA Topology</li> <li>• Specific Factors (Initiation And Elongation) For Protein Synthesis</li> <li>• Maintenance Of mRNA Stability</li> <li>• Maintenance Of</li> </ul>



[illegible]

TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES WHOSE TRANSCRIPTS ARE CHANGED
	Genes associated with trichome-specific metabolic pathways	<ul style="list-style-type: none"> <li>• Changes in terpenoid biosynthesis</li> <li>• Changes in antifeedant and pheromone biosynthesis</li> </ul>	

While trichome polynucleotides and gene products can act alone, combinations of these polynucleotides also affect growth, development and leaf biochemistry. Combinations of trichome polynucleotide(s) and/or gene product(s) with genes or gene products involved in leaf development, hormone responses, or vegetative development are useful because trichome development is integrated with these processes.

#### Use of Promoters of Trichome Genes

Promoters of trichome genes are useful for facilitating transcription of desired polynucleotides, both plant and non-plant in trichomes. For example, extra copies of existing terpenoid synthesis coding sequences can be operably linked to a trichome gene promoter and inserted into a plant to increase the terpenoids in the trichome. Alternatively, trichome promoters can direct expression of non-plant genes or genes from another plant species that can, for instance, lead to new terpenoids being made. The promoters can also be operably linked to antisense copies of coding sequences to achieve down regulation of these gene products in cells.

PATENT  
Attorney Docket No. 2750-1481P

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I.B.3. CHLOROPLAST GENES, GENE COMPONENTS AND PRODUCTS

5 The chloroplast is a complex and specialized organelle in plant cells. Its complexity comes from the fact that it has at least six suborganellar compartments subdivided by double-membrane envelope and internal thylakoid membranes. It is specialized to carry out different biologically important processes including photosynthesis and amino acid and fatty acid biosynthesis. The biogenesis and development of chloroplast from its progenitor (the proplastid) and the conversion of one form of plastid to another (e.g., from chloroplast to amyloplast) depends on several factors that include the developmental and physiological states of the cells.

10 One of the contributing problems that complicate the biogenesis of chloroplast is the fact that some, if not most, of its components must come from the outside of the organelle itself. The import mechanisms must take into account to what part within the different sub-compartments the proteins are being targeted; hence the proteins being imported from the cytoplasm must be able to cross the different internal membrane barriers before they can reach their destinations. The import mechanism must also take into account how to tightly coordinate the interaction between the plastid and the nucleus such that both nuclear and plastidic components are expressed in a synchronous and orchestrated manner. Changes in the developmental and physiological conditions within or surrounding plant cells can consequently change this tight coordination and therefore change how import mechanisms are regulated as well. Manipulation of these conditions and modulation of expression of the import components and their function can have critical and global consequences to the development of the plant and to several biochemical pathways occurring outside the chloroplast. Expression patterns of such genes have been determined using microarray technology.

20  
25 Microarray technology allows monitoring of gene expression levels for thousands of genes in a single experiment. This is achieved by hybridizing labeled fluorescent cDNA pools to glass slides that contain spots of DNA (Schena et al. (1995) Science 270: 467-70). The US Arabidopsis Functional Genomics Consortium (AFGC) has recently made public the results from such microarray experiments conducted with AFGC chips containing about 10,000 non-redundant ESTs, selected from about 37,000 randomly sequenced ESTs generated from mRNA of different tissues and developmental stages.

The sequences of the ESTs showing at least two-fold increases or decreases in a mutant in a mutant (CiA2) of *Arabidopsis thaliana*, that is distributed in chloroplast biogenesis relative to wild type grown in the same conditions were identified, compared to the Ceres full length cDNA and genomic sequence databanks, and equivalent Ceres clones identified. The MA\_diff and/or AFLP\_diff table reports the results of this analysis, indicating those Ceres clones which are up or down regulated over controls, thereby indicating the Ceres clones that are involved in the import of proteins to chloroplast and chloroplast biogenesis.

Examples of genes and gene products that are involved in the import of proteins to chloroplast are shown in the Reference, Sequence, Protein Group, and Protein Group Matrix tables. While chloroplast protein import polynucleotides and gene products can act alone, combinations of these polynucleotides also affect growth and development. Useful combinations include different chloroplast protein import responsive polynucleotides and/or gene products that have similar transcription profiles or similar biological activities, and members of the same or functionally related biochemical pathways. Whole pathways or segments of pathways are controlled by transcription factor proteins and proteins controlling the activity of signal transduction pathways. Manipulation of one or more chloroplast protein import gene activities are useful to modulate the biological processes and/or phenotypes listed below. Chloroplast protein import responsive genes and gene products can act alone or in combination. Useful combinations include chloroplast protein import responsive genes and/or gene products with similar transcription profiles, similar biological activities, or members of the same or functionally related biochemical pathways. Here, in addition to polynucleotides having similar transcription profiles and/or biological activities, useful combinations include polynucleotides that may have different transcription profiles but which participate in common or overlapping pathways. Whole pathways or segments of pathways are controlled by transcription factor proteins and proteins controlling the activity of signal transduction pathways. Therefore, manipulation of such protein levels is especially useful for altering phenotypes and biochemical activities of plants. Manipulation of one or more chloroplast protein import gene activities are useful to modulate the biological processes and/or phenotypes listed below.

Such chloroplast protein import responsive genes and gene products can function to either increase or dampen the above phenotypes or activities in response to changes in the regulation of import mechanisms. Further, promoters of chloroplast protein transport responsive

genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by chloroplast protein transport or any of the following phenotypes or biological activities below. Further, any desired sequence can be transcribed in similar temporal, tissue, or environmentally specific patterns as the chloroplast protein transport responsive genes when the desired sequence is operably linked to a promoter of a chloroplast protein transport responsive gene. The MA\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: Chloroplast (relating to SMD 8093, SMD 8094)). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Chloroplast genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### Chloroplast Genes Identified By Cluster Analyses Of Differential Expression

##### Chloroplast Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Chloroplast genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID Chloroplast (relating to SMD 8093, SMD 8094) of the MA\_diff and/or AFLP\_diff table(s).

##### Chloroplast Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Chloroplast genes. A group in the MA\_clust is considered a Chloroplast pathway or network if the group comprises a cDNA ID that also

appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

5

Chloroplast Genes Identified By Amino Acid Sequence Similarity

Chloroplast genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Chloroplast genes. Groups of Chloroplast genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Chloroplast pathway or network is a group of proteins that also exhibits Chloroplast functions/utilities.

B.3.a. USE OF CHLOROPLAST PROTEIN IMPORT RESPONSIVE GENES  
TO MODULATE PHENOTYPES

Chloroplast protein import responsive genes and gene products are useful to or modulate one or more of the following phenotypes:

- Growth
  - Roots
  - Stems
  - Leaves
  - Development
    - Plastid Biogenesis
    - Plastid Division
    - Plastid Development
    - Thylakoid Membrane Structures
  - Differentiation
    - Plastid/Chloroplast Differentiation
- Photosynthesis
  - Carbon Dioxide Fixation

- Transport
  - Transcription/Translation Regulation Within Transport Complex
  - Phosphate Translocation
  - Targeted Starch Deposition And Accumulation
- Biosynthesis Of Essential Compounds
  - Lipid Biosynthesis
  - Riboflavin Biosynthesis
  - Carotenoid Biosynthesis
  - Aminoacid Biosynthesis

To improve any of the phenotype(s) above, activities of one or more of the chloroplast protein import responsive genes or gene products can be modulated and the plants tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266) and/or screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and visually inspected for the desired phenotype or metabolically and/or functionally assayed according to Saito et al. (1994, Plant Physiol. 106: 887-95), Takahashi et al (1997, Proc. Natl. Acad. Sci. USA 94: 11102-07) and Koprivova et al. (2000, Plant Physiol. 122: 737-46).

#### B.3.b. USE OF CHLOROPLAST PROTEIN IMPORT-RESPONSIVE GENES TO MODULATE BIOCHEMICAL ACTIVITIES

The activities of one or more of the chloroplast protein import responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

GENERAL CATEGORY	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Cell Growth and	• Regulation of Leaf	Reinbothe et al. (1997) Proc.



GENERAL CATEGORY	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Differentiation	Development Including Photosynthetic Apparatus	Natl. Acad. Sci. USA. 94:8890-8894 Eggink and Hooper (2000) J. Biol. Chem. 275:9087-9090 Jagtap et al. (1998) J Exptl Botany 49:1715-1721
	<ul style="list-style-type: none"> <li>Regulation of Plastid Biogenesis and Plastid Division</li> </ul>	Lawrence and Kindle (1997) J. Biol. Chem. 272:20357- 20363 Lahiri and Allison (2000) Plant Physiol. 123:883-894
	<ul style="list-style-type: none"> <li>Development of Plastid Inner/Outer and thylakoid Membrane Structures</li> </ul>	Kouranov et al. (1999) J. Biol. Chem. 274:25181- 25186 Jackson et al. (1998) J. Biol. Chem. 273:16583-16588 Li and Chen (1997) J. Biol. Chem. 272:10968-10974 Lawrence and Kindle (1997) J. Biol. Chem. 272:20357- 20363 Silva-Filho et al. (1997) J. Biol. Chem. 272:15264- 15269
	<ul style="list-style-type: none"> <li>Regulation of transcription and/or translation related to</li> </ul>	May and Soll (2000) Plant Cell 12:53-63 Caliebe et al. (1997) EMBO

GENERAL CATEGORY	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	maintenance of stability of protein-protein interaction within transport complex	J. 16:7342-7350
Physiology	<ul style="list-style-type: none"> <li>• Modulation of Photosynthesis</li> </ul>	Sung and Krieg (1979) Plant Physiol 64: 852-56
	<ul style="list-style-type: none"> <li>• Regulation of Lipid Biosynthesis</li> </ul>	Bourgis et al. (1999) Plant Physiol. 120:913-922 Reverdatto et al. (1999) Plant Physiol. 119:961-978 Roesler et al. (1997) Plant Physiol. 113:75-81
	<ul style="list-style-type: none"> <li>• Regulation of Riboflavin (Vitamin B) biosynthesis</li> </ul>	Jordan et al. (1999) J. Biol. Chem. 274:22114-22121
	<ul style="list-style-type: none"> <li>• Regulation of phosphate translocation across chloroplast membrane</li> </ul>	Flugge (1999) Annu. Rev. Plant Physiol. Plant Mol. Biol. 50:27-45  Silva-Filho et al. (1997) J. Biol. Chem. 272:15264-15269
	<ul style="list-style-type: none"> <li>• Regulation of targeted starch depostion and accumulation</li> </ul>	Yu et al. (1998) Plant Physiol. 116:1451-1460
	<ul style="list-style-type: none"> <li>• Modulation of protein targeting and translocation across chloroplast membrane</li> </ul>	Summer and Cline (1999) Plant Physiol. 119:575-584 Dabney-Smith et al. (1999) J. Biol. Chem. 274:32351-

GENERAL CATEGORY	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
		32359 Hinnah et al. (1997) EMBO J. 16:7351-7360
	<ul style="list-style-type: none"> <li>Regulation of carotenoid biosynthesis</li> </ul>	Bonk et al. (1996) Plant Physiol. 111:931-939
	<ul style="list-style-type: none"> <li>Regulation of amino acid biosynthesis</li> </ul>	Flugge (1999) Annu. Rev. Plant Physiol. Plant Mol. Biol. 50:27-45
	<ul style="list-style-type: none"> <li>Regulation of secondary metabolism</li> </ul>	Flugge (1999) Annu. Rev. Plant Physiol. Plant Mol. Biol. 50:27-45
Signal Transduction	<ul style="list-style-type: none"> <li>Regulation of gene transcriptional activity specific to chloroplast protein import</li> </ul>	Chen et al. (2000) Plant Physiol. 122:813-822. Macasev et al. (2000) Plant Physiol. 123:811-816 .
	<ul style="list-style-type: none"> <li>Regulation of protein target signal cleavage and protein degradation</li> </ul>	Lang et al. (1998) J. Biol. Chem. 273:30973-30978 Jackson et al. (1998) J. Biol. Chem. 273:16583-16588 Richter and Lamppa (1998) Proc. Natl. Acad. Sci. USA. 95:7463-7468
	<ul style="list-style-type: none"> <li>Regulation of ion channel conformation</li> </ul>	Van der Wijngaard and Vredenberg (1999) J. Biol.

GENERAL CATEGORY	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	and activity	Chem. 274:25201-25204
	<ul style="list-style-type: none"> <li>Regulation of kinase and phosphatases synthesis and activity</li> </ul>	Waegemann and Soll (1996) J. Biol. Chem. 271:6545-6554 Li et al. (2000) Science 287:300-303 Muller et al. (2000) J. Biol. Chem. 275:19475-19481
	<ul style="list-style-type: none"> <li>Modulation of Molecular Chaperone and Other Protein Folding Activity</li> </ul>	Bonk et al. (1996) Plant Physiol. 111:931-939 Walker et al. (1996) J. Biol. Chem. 271:4082-4085 Kessler and Blobel (1996). Proc. Natl. Acad. Sci. USA 93:7684-7689 Jackson et al. (1998) J. Biol. Chem. 273:16583-16588

Other biological activities that can be modulated by the chloroplast protein import responsive genes and gene products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table.

- 5 Chloroplast protein import responsive genes are characteristically differentially transcribed in response to fluctuating chloroplast protein import levels or concentrations, whether internal or external to an organism or cell. The MA\_diff and/or AFLP\_diff reports the changes in transcript levels of various chloroplast protein import responsive genes that are differentially expressed among the mutants and the wild type.

Profiles of some of these chloroplast protein import responsive genes are shown in the Table below together with examples of the kinds of associated biological activities.

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
Up regulated transcripts	<p>Responders to defective chloroplast protein import</p> <p>Genes induced by defective import</p>	<ul style="list-style-type: none"> <li>• Chloroplast protein import regulation</li> <li>• Chloroplast protein import and transport</li> <li>• Chloroplast import metabolism</li> <li>• Synthesis of secondary metabolites and/or proteins</li> <li>• Modulation of chloroplast import response transduction pathways</li> <li>• Changes in chloroplast membranes</li> <li>• Specific gene transcription initiation</li> <li>• Chloroplast and</li> </ul>	<ul style="list-style-type: none"> <li>• Transporters</li> <li>• Metabolic enzymes</li> <li>• Change in cell membrane structure and potential</li> <li>• Kinases and phosphatases</li> <li>• Transcription activators</li> <li>• Change in chromatin structure and/or localized DNA topology</li> <li>• Redox control</li> <li>• Metabolic enzymes concerned with chloroplast biochemistry</li> <li>• Organelle gene expression and translation</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
		non-chloroplast metabolic pathways	
Down-regulated transcripts	<p>Responders to defective chloroplast protein import.</p> <p>Genes repressed by defective chloroplast protein import</p> <p>Genes with unsTable mRNAs when chloroplast import is defective</p> <p>Genes with discontinued expression or unsTable mRNA in presence of chloroplast protein import</p>	<ul style="list-style-type: none"> <li>• Regulation of chloroplast protein import pathways released</li> <li>• Chloroplast protein import and transport</li> <li>• Chloroplast import metabolism</li> <li>• Changes in pathways and processes operating in chloroplasts</li> <li>• Changes in organelle membranes</li> <li>• Loss of organelle gene expression, RNA and protein synthesis</li> <li>• Changes in metabolism other</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription factors</li> <li>• Change in protein structure by phosphorylation (kinases) or dephosphorylation (phosphatases)</li> <li>• Change in chromatin structure and/or DNA topology</li> <li>• Stability factors for protein mRNA synthesis and degradation</li> <li>• Organelle transcription and translation proteins</li> <li>• Metabolic enzymes</li> </ul>

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TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
		than chloroplast protein import pathways • Chloroplast import metabolism	

#### Use of Promoters of Chloroplast Genes

Promoters of Chloroplast genes are useful for transcription of any desired polynucleotide or plant or non-plant origin. Further, any desired sequence can be transcribed in a similar temporal, tissue, or environmentally specific patterns as the Chloroplast genes where the desired sequence is operably linked to a promoter of a Chloroplast gene. The protein product of such a polynucleotide is usually synthesized in the same cells, in response to the same stimuli as the protein product of the gene from which the promoter was derived. Such promoter are also useful to produce antisense mRNAs to down-regulate the product of proteins, or to produce sense mRNAs to down-regulate mRNAs via sense suppression

I.C. REPRODUCTION GENES, GENE COMPONENTS AND PRODUCTS

I.C.1. REPRODUCTION GENES, GENE COMPONENTS AND PRODUCTS

5       Reproduction genes are defined as genes or components of genes capable of modulating any aspect of sexual reproduction from flowering time and inflorescence development to fertilization and finally seed and fruit development. These genes are of great economic interest as well as biological importance. The fruit and vegetable industry grosses over \$1 billion USD a year. The seed market, valued at approximately \$15 billion USD annually, is even more lucrative.

10       Expression of many reproduction genes and gene products is orchestrated by internal programs or the surrounding environment of a plant, as described below. These genes and/or products have great importance in determining traits such as fruit and seed yield. Examples of such reproduction genes and gene products are shown in the Reference, Sequence, Protein Group, Protein Group Matrix tables, Knock-in, Knock-out, MA-diff and MA-clust. The biochemical functions of the protein products of many of these genes determined from  
15       comparisons with known proteins are also given in the Reference tables.

Reproduction Genes Identified by Phenotypic Observation

20       Reproduction genes were discovered and characterized from a much larger set of genes by experiments designed to find genes that cause phenotypic changes in flower, silique, and seed morphology. In these experiments, reproduction genes were identified by either (1) ectopic expression of a cDNA in a plant or (2) mutagenesis of the plant genome. The plants were then cultivated and phenotypes, which varied from the parental "wild-type", were observed.

25       One particular example of reproductive genes are those that are regulated by AP2. AP2 is a transcription factor that regulates many genes, both as a repressor of some genes and as an activator of others. Some of these genes are those which establish the floral meristem or those which regulate floral organ identity and development. As such, AP2 has an effect on reproduction. This is, loss of AP2 activity is correlated with decreased male and female reproduction. AP2 is also known to have an effect on seed mass, and therefore on yield. That is,  
30       overexpression of AP2 is correlated with smaller seeds or seedless fruit while repression of AP2 correlates with larger seeds (see, e.g. US Patent No.: 5,994,622).



Another example of reproduction genes are those that are regulated by PISTILLATA (PI). PI is a transcription factor that regulates many genes both as a repressor and activator. Some of these genes are those which regulate floral organ identity and development, in conjunction with other transcription factors such as AP2 and AGAMOUS. As such, PI has an effect on reproduction in that loss of PI activity is correlated with decreased male reproduction. PI is also known to have an effect on carpel number, and therefore potentially on ovule/seed number and yield. Specifically, repression of PI results in increased carpel number and therefore ovule number.

Yet another example of reproductive genes are those that are regulated by MEDEA (MEA). MEA is a SET-domain containing protein that associates with other proteins to form complexes that affect chromatin structure and therefore gene expression. As such, loss of MEA function is correlated with global gene activation and repression leading to many phenotypes including decreased female reproduction and therefore reduced seed set and yield.

In the characterization of these and other reproduction genes, the following phenotypes were scored:

I. Flower

- Size
  - Large
  - Small
- Shape
  - Abnormal organ numbers
  - Agamous
  - AP-2 like
- Color
- Number
- Fused Sepals

II. Silique

- Size
- Seed number
  - Reduced
  - Absent

- Seed color

The identified genes regulating reproduction are identified in the Knock-in and Knock-out Tables.

5 Reproduction Genes Identified by Differential Expression

Reproduction genes were also identified in experiments designed to discover genes whose mRNA products were in different concentrations in whole flowers, flower parts, and siliques relative to the plant as a whole. The MA\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108473, 108474, 108429, 108430, 108431, 108475, 108476, 108501). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Reproduction genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff tables with a "+" or "-" indication

Reproduction Genes Identified By Cluster Analyses Of Differential Expression

Reproduction Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Reproduction genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108473, 108474, 108429, 108430, 108431, 108475, 108476, 108501 of the MA\_diff and/or AFLP\_diff table(s).

Reproduction Genes Identified By Correlation To Genes That Cause Physiological Consequences

5 Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Reproduction genes. A group in the MA\_clust is considered a Reproduction pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

#### Reproduction Genes Identified By Amino Acid Sequence Similarity

10 Reproduction genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Reproduction genes. Groups of Reproduction genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Reproduction pathway or network is a group of proteins that also exhibits Reproduction functions/utilities.

15 It is assumed that the reproduction genes differentially expressed in floral parts and seeds are concerned with specifying flowers and seeds and their functions, and therefore modulations of such genes produce variant flowers and seeds.

20 Reproductive genes and gene products can function to either increase or dampen the phenotypes, biochemical activities and transcription profiles, either in response to changes of internal plant programs or to external environmental fluctuations.

#### C.1.a. USE OF REPRODUCTION GENES, GENE COMPONENTS AND PRODUCTS TO MODULATE PHENOTYPES

25 The reproduction genes of the instant invention, when mutated or activated differently, are capable of modulating one or more processes of flower, seed and fruit development. They are thus useful for improving plants for agriculture and horticulture and for providing seeds with a better chemical composition for diverse industries including the food, feed and chemical industries. Reproduction genes, gene components and products are useful to alter the following traits and properties of plants:

##### I. Development

##### A. Flowering time and number of inflorescences

B. Flower development

- (a) Anther
- (b) Stamen
- (c) Pollen
- (d) Style
- (e) Stigma
- (f) Ovary
- (g) Ovule
- (h) Gametes

C. Pollination and Fertilization

- (a) Sporogenesis
- (b) Gametogenesis
- (c) Zygote formation
- (d) Embryo development
- (e) Endosperm development
- (f) Male sterility, hybrid breeding systems and heterosis

II. Cellular Properties:

- A. Cell size
- B. Cell shape
- C. Cell death
- D. Cell division
- E. Cell elongation
- F. Cell differentiation
- G. Meiosis

III. Organ Characteristics:

- A. Flowers
  - (1) Receptacle
  - (2) Sepals, Petals, and Tepals
    - a. Color

- b. Shape
- c. Size
- d. Number
- e. Petal Drop

(3) Androecium

Stamen

(i) Anther

- 1. Size
- 2. Pollen

a. Sterile

b. Size, shape, weight, color

3. Number

(ii) Filament

- 1. Size

(4) Gynoecium

a. Carpel

(i) Ovary

(ii) Number

(iii) Length

b. Style

(i) Stigma

(ii) Ovule

Size, shape, number

(5) Pedicel and Peduncle

a. Size

b. Shape

B. Seeds

- 1. Placenta
- 2. Embryo
- 3. Cotyledon
- 4. Endosperm

5. Suspensor
6. Seed coat (testa)
7. Aleurone
8. Development, including Apomixis (gametophytic, apospory, diplospory)
9. Dormancy
10. Germination

C. Fruits

1. Pericarp – thickness, texture
  - (a) Exocarp
  - (b) Mesocarp
  - (c) Endocarp
2. Development
  - (a) Seed set
  - (b) Fruit set
  - (c) False fruit
  - (d) Fruit elongation and maturation
  - (e) Dehiscence
3. Fruit drop

IV. Plant Seed Yield

1. Increased biomass
2. Better Harvest Index
3. Attraction of favorable insects
4. Better seed quality
5. Better yield of constituent chemicals

V. Plant Population Features:

A. Architecture

1. Shade avoidance
2. Planting density

To regulate any of the phenotype(s) above, activities of one or more of the reproduction genes or gene products can be modulated in an organism and tested by screening for the desired trait. Specifically, the gene, mRNA levels or protein levels can be altered in a plant using the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (Methods Mol. Biol. 82:259-266 (1998)) and/or screened for variants as in Winkler et al. (Plant Physiol 118:743-50 (1998)) and visually inspected for the desired phenotype or metabolically and/or functionally assayed.

#### C.1.b. USE OF REPRODUCTION GENES TO MODULATE BIOCHEMICAL ACTIVITIES

The activities of one or more of the reproduction genes can be modulated to change biochemical or metabolic activities and/or pathways such as those examples noted below. Such biological activities can be measured according to the citations included in the Table below:

FUNCTION/PROCESS	EXAMPLES OF BIOCHEMICAL/MOLECULAR ACTIVITIES	Reference AND ASSAY
Metabolism	<p>Energy production and conversion</p> <ul style="list-style-type: none"> <li>- Glucosyl-transferase (CLONE_ID 1040)</li> <li>- Heme-binding protein (putative cytochrome B5) (CLONE_ID 3743)</li> </ul> <p>Storage protein synthesis</p> <p>Inorganic ion transport and metabolism</p> <ul style="list-style-type: none"> <li>- Peroxidase</li> </ul>	<p>Ap Rees, T. (1974). In "Plant Biochemistry. Biochemistry, Series One", Vol. 11. (H.L. Kornberg and D.C. Phillips, eds.), Butterworths, London.</p> <p>Juliano, B.O. and Varner, J.E. (1969). <i>Plant Physiol.</i> 44, 886-892.</p> <p>Bewley et al. (1993). <i>Plant Physiol. Biochem.</i> 31, 483-490.</p> <p>Hills, M. J. and Beevers, H. (1987). <i>Plant Physiol.</i> 84,</p>

[illegible]



	<p>(CLONE_ID 7530)</p> <p>Lipid metabolism</p> <ul style="list-style-type: none"> <li>- Branched chain <math>\alpha</math>-ketoacid dehydrogenase E2 subunit</li> </ul> <p>(CLONE_ID 25116)</p> <ul style="list-style-type: none"> <li>- Acyl carrier protein-1</li> </ul> <p>(CLONE_ID 14291)</p> <ul style="list-style-type: none"> <li>- Lipid metabolic enzymes</li> </ul> <p>Secretion</p> <ul style="list-style-type: none"> <li>- Sensor protein RcsC-like</li> </ul> <p>(CLONE_ID 16461)</p> <ul style="list-style-type: none"> <li>- Signal recognition particle</li> </ul> <p>RP54 (CLONE_ID 22158)</p>	<p>Chrispeels, M. J. and Jones, R. L. (1980/81). <i>Isr. J. Bot.</i> 29, 222-245.</p> <p>Gould, S. E. B., and Rees, D. A. (1964). <i>J. Sci. Food Agric.</i> 16, 702-709.</p>
Modulate floral organ number	<p>Transcriptional control</p> <p>ANT (AP2-domain) DNA binding protein</p> <p>SUP (Zinc finger)</p>	<p>Elliot et al. (1996). <i>Plant Cell</i> 8, 155-168.</p> <p>Sakai et al. (2000). <i>Plant Cell</i> 12, 1607-1618.</p> <p>Jacobsen and Meyerowitz (1997). <i>Science</i> 277, 1100-1103.</p>
Floral organ size	<p>Transcriptional control</p> <p>ANT (AP2-domain) DNA binding protein</p>	<p>Mizukami et al. (2000). <i>PNAS</i> 97, 942-947.</p> <p>Krizek (1999). <i>Developmental Genetics</i> 25,</p>

		224-236.
Female organ number/Floral meristem size	Membrane receptor kinase signal transduction CLV1 (LRR domain and kinase domain) receptor CLV2 (LRR domain) receptor  CLV3 (Receptor ligand)	Clark and Meyerowitz (1997). Cell 89, 575-585 Jeong et al. (1999). Plant Cell 11, 1925-1934. Fletcher et al. (1999). Science 283, 1911-1914.
Female reproduction	DNA binding protein AG (MADS domain) DNA binding protein	Yanofsky et al. (1990). Nature 346, 35-39.
Female reproduction	Signal transduction CTR1 (Raf kinase)	Kieber et al. (1993). Cell 72, 427-441.
Male organ number	DNA methylation MET1 (DNA methyltransferase)	Jacobsen and Meyerowitz (1997). Science 277, 1100- 1103.
Seed size control	DNA binding protein AP2 (AP2 domain) RAP2 (AP2 domain)	Jofuku et al. (1994). Plant Cell 6, 1211-1225. US Patent #6,093,874; #5,994,622
Seed size control	Polycomb group protein complex FIE, FIS2, MEA	Luo et al. (2000). PNAS 97, 10637-10642.

Seed size control	DNA methylation MET1	Scott et al. (2000). Development 127, 2493-2502. Vinkenoog et al. (2000). Plant Cell 12, 2271-2282. Luo et al. (2000). PNAS 97, 10637-10642.
Embryo development/Embryo viability	CAAT box binding complex LEC1/HAP3 HAP2, HAP5	Lotan et al. (1998). Cell 93, 1195. US Patent #6,235,975
Embryo development/Seed dormancy	DNA binding proteins ABI4 (AP2 domain)  FUS3 (B3 domain) VP1 (B3 domain)	Finkelstein et al. (1998). Plant Cell 10, 1043-1054. Luerssen et al. (1998). Plant J. 15, 755-764.
Embryo development	Signal transduction ABI1, ABI2 [Serine/threonine protein phosphatase 2C (PP2C)]	Leung et al. (1994). Science 264, 1448-1452. Leung et al. (1997). Plant Cell 9, 759-771.
Endosperm development	Chromatin level control of gene activity Polycomb complex; FIE, MEA, FIS2	Ohad et al. (1996). PNAS 93, 5319-5324. US Patent #6,229,064 Kiyosue et al. (1999). PNAS 96, 4186-4191.

		Grossniklaus et al. (1998). Science 280, 446-450. Chaudhury et al. (1997) PNAS 94, 4223-4228.
Integument development/Seed coat development	DNA binding AP2, ANT (AP2 domain)  BEL1 (Homeodomain)	Jofuku et al. (1994). Plant Cell 6, 1211-1225. Klucher et al. Plant Cell 8, 137-153. Reiser et al. (1995). Cell 83, 735-742.
Anthocyanin production	Secondary transporter TT12 (MATE; multidrug and toxic compound extrusion)	Debeaujon et al. (2001). Plant Cell 13, 853-872.
Anthocyanin production	DNA binding protein TT8 (Basic helix-loop-helix domain)	Nesi et al. (2000). Plant Cell 12, 1863-1878.
Fruit development	Chromatin level control of gene activity Polycomb complex; FIE, MEA, FIS2	Ohad et al. (1996). PNAS 93, 5319-5324. Kiyosue et al. (1999). PNAS 96, 4186-4191. Grossniklaus et al. (1998). Science 280, 446-450. Chaudhury et al. (1997) PNAS 94, 4223-4228.
Fruit size control	Signal transduction FW2.2 (c-Ras P21)	Frary et al. (2000). Science 289, 85-88.
Fruit development/Pod shattering	Transcriptional control SHP1, SHP2, FUL (MADS domain) DNA binding	Liljegren et al. (2000). Nature 404, 766-770.

	proteins	Ferrandiz et al. (2000). Science 289, 436-438..
Transcription and Posttranscription	<p>Transcription</p> <ul style="list-style-type: none"> <li>- SRF-domain AGL11 (CLONE_ID 32791)</li> <li>- AP2-domain containing protein (CLONE_ID 332)</li> <li>- Myb-DNA binding protein (CLONE_ID 94597)</li> </ul> <p>Transcription factors</p> <p>Signal transduction mechanisms</p> <ul style="list-style-type: none"> <li>- Protein-kinases</li> <li>- Phosphatases</li> <li>- meiosis proteins</li> <li>- Chromatin remodeling proteins</li> <li>- Chaperones</li> <li>- Chalcone synthase</li> <li>- Putative Ser/Thr protein kinase (CLONE_ID 31383)</li> <li>- ER6-like protein (implicated in ethylene signal transduction) (CLONE_ID 7474)</li> </ul>	<p>Delseny, M. et al. (1977). Planta 135, 125-128.</p> <p>Lalonde, L. and Bewley, J. D. (1986). J. Exp. Bot. 37, 754-764.</p> <p>Walling, L. et al. (1986). PNAS 83, 2123-2125.</p> <p>Okamuro, J. K. and Goldberg, R. B. (1989). In "Biochemistry of Plants, Vol 15." Academic Press, Inc.</p> <p>Wong, J. et al. (1995). Genes Dev. 9, 2696-2711.</p> <p>Dimitrov et al. (1994). J. Cell Biol. 126, 591-601.</p> <p>Landsberger, N. and Wolffe, A. P. (1997). EMBO J. 16, 4361-4373.</p> <p>Bogdanove, A. J. and Martin, G. G. (2000). PNAS 97, 8836-8840.</p> <p>Zhu, H. et al. Science July 26, 2001: 10.1126/science.1062191 (Reports).</p>

	<p>Translation, ribosomal structure and biogenesis</p> <ul style="list-style-type: none"> <li>- Ribosomal proTein S15A (CLONE_ID 17466)</li> <li>- Translation initiation factor (CLONE_ID 103464)</li> </ul> <p>Posttranslational modification, protein turnover, chaperones</p> <ul style="list-style-type: none"> <li>- DnaJ-domain containing protein (CLONE_ID 4150)</li> <li>- Cyclophilin-like protein (CLONE_ID 35643)</li> </ul>	
Cell division and Repair	<p>Cell division and chromosome partitioning</p> <ul style="list-style-type: none"> <li>- Protein of unknown function with tropomyosin-, myosin tail- and filament-domains (CLONE_ID 15546)</li> <li>- Actin-1 (CLONE_ID 25785)</li> </ul> <p>DNA replication,</p>	<p>Rogan, P. G. and Simon, E. W. (1975). New Phytol. 74, 273-275.</p> <p>Morahashi, Y. and Bewley, J. D. (1980). Plant Physiol 66, 70-73.</p> <p>Morahashi, Y. et al. (1981). Plant Physiol. 68, 318-323.</p> <p>Morahashi, Y. (1986). Physiol. Plant. 66, 653-658.</p> <p>Zlatanova, J. et al. (1987). Plant Mol. Biol. 10, 139-144.</p>

	<p>recombination and repair</p> <ul style="list-style-type: none"> <li>- Proliferating cell nuclear antigen-1 (axillary protein, DNA polymerase I delta) (CLONE_ID 28554)</li> <li>- AAA-type ATPase, cdc48 (CLONE_ID 100292)</li> </ul> <p>Cell envelope biogenesis, outer membrane</p> <ul style="list-style-type: none"> <li>- dTDP-D-glucose 4,6-dehydratase (CLONE_ID 28597)</li> <li>- Putative cinnamoyl-CoA reductase (CLONE_ID 109228)</li> </ul>	<p>Zlatanova, J. and Ivanov, P. (1988). Plant Sci. 58, 71-76.</p>
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Other biological activities that are modulated by the reproductive genes and gene products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table, for example.

C.1.c. USE OF REPRODUCTION GENES, GENE COMPONENTS AND PRODUCTS  
TO MODULATE TRANSCRIPTION LEVELS

Reproduction genes are characteristically differentially transcribed in response to cell signals such as fluctuating hormone levels or concentrations, whether internal or external to an organism or cell. Many reproduction genes belong to networks or cascades of genes under the control of regulatory genes. Thus some reproduction genes are useful to modulate the expression of other genes. Examples of transcription profiles of reproduction genes are described in the Table below with associated biological activities. "Up-regulated" profiles are those where the mRNA transcript levels are higher in flowers, flower parts or siliques as compared to the plant as a whole. "Down-regulated" profiles represent higher transcript levels in the whole plant as compared to flowers, flower parts or siliques alone.

TRANSCRIPT LEVELS	TYPE OF GENES WITH ALTERED ACTIVITY	PHYSIOLOGICAL CONSEQUENCES OF ALTERING GENE EXPRESSION	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENES WITH ALTERED EXPRESSION
Up Regulated Transcripts Flower Reproduction Genes	Genes that control flower differentiation, number and size  Genes that promote petal, stamen and carpel formation  Genes controlling flower-specific metabolism such as petal pigments	Flowers form from flower meristem  Floral organs mature  Flavonoid pathways induced	Transcription Factors Signal transduction Membrane Structure Protein kinases Phosphatases Meiosis proteins Chromatin remodeling proteins Chaperones Chalcone synthase  Amino acid transport



TRANSCRIPT LEVELS	TYPE OF GENES WITH ALTERED ACTIVITY	PHYSIOLOGICAL CONSEQUENCES OF ALTERING GENE EXPRESSION	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENES WITH ALTERED EXPRESSION
	<p>Genes that promote ovule formation</p> <p>Genes that promote fertilization, seed, embryo and endosperm formation</p>		<p>and metabolism</p> <p>Storage protein synthesis</p> <p>Lipid metabolic enzymes</p> <p>Carbohydrate transport and metabolism</p> <p>Starch biosynthesis</p>
<p>AP2</p> <p>Reproduction</p> <p>Genes</p>	<p>Genes activated by AP2 transcription factors</p> <p>Genes that induce petal and stamen formation</p>	<p>Many steps and pathways induced, developmental and metabolic</p> <p>No petals or stamens produced</p>	<p>Proteins associated with:</p> <p>Energy production and conversion</p> <p>Amino acid transport and metabolism</p> <p>Carbohydrate transport and metabolism</p> <p>Lipid metabolism</p> <p>Transcription and</p>

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TRANSCRIPT LEVELS	TYPE OF GENES WITH ALTERED ACTIVITY	PHYSIOLOGICAL CONSEQUENCES OF ALTERING GENE EXPRESSION	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENES WITH ALTERED EXPRESSION
			signal transduction Poor translational modification DNA replication Chromatin remodeling
Down-Regulated Transcripts Flower Reproduction Genes	Genes that repress flower development  Genes that induce stem, leaf and other organ differentiation  Genes that negatively regulate flower specific metabolism  Genes that negatively regulate ovule	Flowers form from flower meristem  Non-floral organs are repressed  Flower-specific pathways are induced	Transcription factors Signal transduction pathways Kinases and phosphatases  Chromatin remodeling proteins

TRANSCRIPT LEVELS	TYPE OF GENES WITH ALTERED ACTIVITY	PHYSIOLOGICAL CONSEQUENCES OF ALTERING GENE EXPRESSION	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENES WITH ALTERED EXPRESSION
AP2 Reproduction Genes	<p>formation, meiosis, fertilization and seed development</p> <p>Genes activated by AP2 transcription factors</p> <p>Genes that induce petal and stamen formation</p>	<p>Many steps and pathways induced, developmental and metabolic</p> <p>No petals or stamens produced</p>	<p>Proteins associated with:</p> <p>Energy production and conversion</p> <p>Amino acid transport and metabolism</p> <p>Carbohydrate transport and metabolism</p> <p>Lipid metabolism</p> <p>Transcription and signal transduction</p> <p>Poor translational modification</p> <p>DNA replication</p> <p>Chromatin remodeling</p>

While polynucleotides and gene products modulating reproduction can act alone, combinations of these polynucleotides also affect growth and development. Useful combinations

include different polynucleotides and/or gene products of the instant invention that have similar transcription profiles or similar biological activities, and members of the same or similar biochemical pathways. In addition, the combination of a polynucleotide and/or gene product(s) capable of modulating reproduction with a hormone responsive polynucleotide, particularly one  
5 affected by gibberellic acid and/or auxin, is also useful because of the interactions that exist between hormone regulated pathways, and development. Here, in addition to polynucleotides having similar transcription profiles and/or biological activities, useful combinations include polynucleotides that may have different transcription profiles but which participate in common or overlapping pathways.

#### Use Of Promoters And Reproduction Genes

Promoter of reproduction genes are useful for transcription of desired polynucleotides, both plant and non-plant. For example, extra copies of carbohydrate transporter genes can be operably linked to a reproduction gene promoter and inserted into a plant to increase the "sink" strength of flowers or siliques. Similarly, reproduction gene promoters can be used to drive transcription of metabolic enzymes capable of altering the oil, starch, protein or fiber of a flower or silique. Alternatively, reproduction gene promoters can direct expression of non-plant genes that can, for instance confer insect resistance specifically to a flower.

I.C.2. OVULE GENES, GENE COMPONENTS AND PRODUCTS

The ovule is the primary female sexual reproductive organ of flowering plants. It contains the egg cell and, after fertilization occurs, contains the developing seed. Consequently, the ovule is at times comprised of haploid, diploid and triploid tissue. As such, ovule development requires the orchestrated transcription of numerous polynucleotides, some of which are ubiquitous, others that are ovule-specific and still others that are expressed only in the haploid, diploid or triploid cells of the ovule.

Although the morphology of the ovule is well known, little is known of these polynucleotides and polynucleotide products. Mutants allow identification of genes that participate in ovule development. As an example, the pistillata (PI) mutant replaces stamens with carpels, thereby increasing the number of ovules present in the flower. Accordingly, comparison of transcription levels between the wild-type and PI mutants allows identification of ovule-specific developmental polynucleotides.

Changes in the concentration of ovule-specific polynucleotides during development results in the modulation of many polynucleotides and polynucleotide products. Examples of such ovule-specific responsive polynucleotides and polynucleotide products are shown in the Reference, Sequence, Protein Group, Protein Group Matrix, AFLP\_diff, MA\_diff and/or AFLP\_diff, and MA\_clust tables. These polynucleotides and/or products are responsible for effects on traits such as fruit production and seed yield.

While ovule-specific developmentally responsive polynucleotides and polynucleotide products can act alone, combinations of these polynucleotides also affect fruit and seed growth and development. Useful combinations include different ovule-specific developmentally responsive polynucleotides and/or polynucleotide products that have similar transcription profiles or similar biological activities, and members of the same or similar biochemical pathways. In addition, the combination of an ovule-specific developmentally responsive polynucleotide and/or polynucleotide product with an environmentally responsive polynucleotide is also useful because of the interactions that exist between development, hormone regulated pathways, stress pathways and nutritional pathways. Here, in addition to polynucleotides having similar transcription profiles and/or biological activities, useful combinations include polynucleotides that may have different transcription profiles but which participate in a common

pathway. The MA\_diff and/or AFLP\_diff, AFLP\_int and AFLP\_diff Table(s) report the transcript levels of the experiment (see EXPT ID: 108595). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Ovule genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### Ovule Genes Identified By Cluster Analyses Of Differential Expression

##### Ovule Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Ovule genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108595 of the MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

##### Ovule Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Ovule genes. A group in the MA\_clust is considered a Ovule pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

##### Ovule Genes Identified By Amino Acid Sequence Similarity

Ovule genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Ovule genes. Groups of Ovule

genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Ovule pathway or network is a group of proteins that also exhibits Ovule functions/utilities.

5 Such ovule-specific developmentally responsive polynucleotides and polynucleotide products can function to either increase or dampen the above phenotypes or activities either in response to transcript changes during ovule development or in the absence of ovule-specific polynucleotide fluctuations. More specifically, ovule-specific developmentally responsive polynucleotides and polynucleotide products are useful to or modulate one or more of the following phenotypes:

- Egg Cell
- Maturation
- for development of parthenogenic embryos
- Metabolism
- Polar nuclei
- Fusion
- for development of parthenogenic endosperm
- Central Cell
- Maturation
- Metabolism
- For alteration of endosperm metabolism
- Synergids
- Maturation
- Programmed cell death
- Nucellus
- Maturation
- Integuments
- Maturation
- Funiculus
- Extension
- for increased seed

- Cuticle
- Maturation
- Tensile properties
  - for increased seed size
- Ovule
- Modulation of ovule senescence
- Shaping
  - for increased seed number

To produce the desired phenotype(s) above, one or more of the ovule-specific developmentally responsive polynucleotides and polynucleotide products can be tested by screening for the desired trait. Specifically, the polynucleotide, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266) and visually inspected for the desired phenotype or metabolically and/or functionally assayed according to Weigel et al. (2000, Plant Physiol 122: 1003-14) and Winkler et al. (1998, Plant Physiol 118: 743-50).

Alternatively, the activities of one or more of the ovule-specific developmentally responsive polynucleotides and polynucleotide products can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

GENERAL CATEGORY	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	ASSAY
Cell Growth and Differentiation	-Programmed Cell Death	Pennell and Lamb (1997) Plant Cell 9, 1157-1168
	- DNA Methylation and	Adams et al. (2000)



GENERAL CATEGORY	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	ASSAY
	Imprinting	Development 127: 2493-502
Organ Growth and Development	-Ovule Growth and Development -Ethylene Response -Megagametophyte Development  -Seed Growth and Development  -Fertilization Independent Seed Development	De Martinis and Mariani (1999) Plant Cell 11: 1061-72 Christensen et al. (1997) Sexual Plant Reproduc 10: 49-64  Scott et al. (1998) Development 125: 3329-41 Ohad et al. (1996) PNAS USA 93: 5319-24 Chaudhury et al. (1997) PNAS USA 94: 4223-28
Signal Transduction	-Ethylene Metabolism  - Protein Remodeling -Sucrose Mobilization and Partitioning -Pollen Tube Adhesion -Jasmonic Acid	DeMartinis and Mariani (1999) Plant Cell 11: 1061-1072 Winkler et al. (1998) Plant Physiol 118: 743- 750

GENERAL CATEGORY	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	ASSAY
	Biosynthesis -	
Senescence and Cell Death	-Apomixis	
Environmental Responses	-Wound and Defense Response Gene Expression  -Stress Response	Epple and Bohlmann (1997) Plant Cell 9: 509- 20 He et al. (1998) Plant J. 14: 55-63

Other biological activities that can be modulated by the ovule-specific developmentally responsive polynucleotides and polynucleotide products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table section.

Ovule-specific developmentally responsive polynucleotides are characteristically differentially transcribed in response to fluctuating developmental-specific polynucleotide levels or concentrations, whether internal or external to a cell. The MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) report the changes in transcript levels of various ovule-specific developmentally responsive polynucleotides in ovules.

These data can be used to identify a number of types of ovule-specific developmentally responsive polynucleotides. Profiles of these different ovule-specific developmentally responsive polynucleotides are shown in the Table below with examples of associated biological activities.

### Use of Promoters of Ovule Genes

5

antisense mRNAs to down-regulate the product of proteins, or to produce sense mRNAs to down-regulate mRNAs via sense suppression.

I.C.3. SEED AND FRUIT DEVELOPMENT GENES, GENE COMPONENTS  
AND PRODUCTS

5 The ovule is the primary female sexual reproductive organ of flowering plants. At maturity it contains the egg cell and one large central cell containing two polar nuclei encased by two integuments that, after fertilization, develops into the embryo, endosperm, and seed coat of the mature seed, respectively. As the ovule develops into the seed, the ovary matures into the fruit or silique. As such, seed and fruit development requires the orchestrated transcription of numerous polynucleotides, some of which are ubiquitous, others that are embryo-specific and still others that are expressed only in the endosperm, seed coat, or fruit. Such genes are termed fruit development responsive genes.

10 Changes in the concentration of fruit-development responsive polynucleotides during development results in the modulation of many polynucleotides and polynucleotide products. Examples of such fruit development responsive polynucleotides and polynucleotide products relative to leaves and floral stem are shown in the Reference, Sequence, Protein Group, Protein Group Matrix, AFLP\_diff, MA\_diff and/or AFLP\_diff, MA\_clust, Knock-in and Knock-out tables. The polynucleotides were discovered by isolating fruits at developmental stages from Arabidopsis wild-type ecotype "Wassilewskija", and measuring the mRNAs expressed in them relative to those in a leaf and floral stem sample. These polynucleotides and/or products are responsible for effects on traits such as seed size, seed yield, seed composition, seed dormancy, fruit ripening, fruit production, and pod shattering.

20 While fruit development responsive polynucleotides and polynucleotide products can act alone, combinations of these polynucleotides also affect fruit and seed growth and development. Useful combinations include different polynucleotides and/or polynucleotide products that have similar transcription profiles or similar biological activities, and members of the same or functionally similar biochemical pathways. In particular, modulation of transcription factors and/or signal transduction pathways are likely to be useful for manipulating whole pathways and hence phenotypes. In addition, the combination of ovule-developmentally responsive polynucleotides and/or polynucleotide products with environmentally responsive polynucleotides is also useful because of the interactions that exist between development, hormone regulated pathways, stress and pathogen induced pathways and nutritional pathways. Here, useful

combinations include polynucleotides that may have different transcription profiles, and participate in common or overlapping pathways but combine to produce a specific, phenotypic change.

Such fruit development responsive polynucleotides and polynucleotide products can function to either increase or dampen the above phenotypes or activities either in response to transcript changes in fruit development or in the absence of fruit development polynucleotide fluctuations.

The MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) report the transcript levels of the experiment (see EXPT ID: 108436, 108437, 108438). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Fruit genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the AFLP\_diff and/or MA\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### Fruit Genes Identified By Cluster Analyses Of Differential Expression

#### Fruit Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Fruit genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108436, 108437, 108438 of the MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

#### Fruit Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Fruit genes. A group in the MA\_clust is considered

a Fruit pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

Fruit Genes Identified By Amino Acid Sequence Similarity

5 Fruit genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Fruit genes. Groups of Fruit genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Fruit pathway or network is a group of proteins that also exhibits Fruit functions/utilities.

10 - Use of fruit development responsive genes to modulate phenotypes

15 Manipulation of the polynucleotides in the mature ovule, developing embryo, endosperm, seed coat and fruit enables many features of seed and fruit to be improved including the following:

- 20 - Female fertility, megasporogenesis, embryo and endosperm development, ovule size, endosperm size, embryo size, seed size, seed yield, seed protein, seed oil, seed starch, seed cell number, cell size, seed coat development, organ size, dormancy and acquisition of desiccation tolerance, seed storage and longevity, seed germination, apomixis, production of seedless fruit and vegetables and hybrid seed production.

25 To improve any of the phenotype(s) above, activities of one or more of the fruit development responsive polynucleotides and polynucleotide products can be modulated and the plants can be tested by screening for the desired trait. Specifically, the polynucleotide, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266) and visually inspected for the desired  
30 phenotype or metabolically and/or functionally assayed.

**Use of fruit development responsive genes to modulate biochemical activities**

The activities of one or more of the fruit-expressed polynucleotides and polynucleotide products can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological changes can be achieved and measured according to citations such as the following:

1. Winkler et al. (1998). Plant Physiol. 118, 743-750
2. Weigel et al. (2000). Plant Physiol. 122, 1003-1014
3. Cosgrove (1997). Plant Cell 9, 1031-1041
4. Jacobs (1997). Plant Cell 9, 1021-1029
5. Reismeier et al. (1994). EMBO J. 13, 1-7
6. Carland et al. (1999). Plant Cell 11, 2123-2138
7. Cheng et al. (1996). Plant Cell 8, 971-983
8. Weber et al. (1995). Plant Cell 7, 1835-1846
9. Leyser and Furner (1992). Development 116, 397-403
10. Hayashi et al. (1998). Plant Cell 10, 183-196.
11. Pyke (1999). Plant Cell 11, 549-556
12. Lotan et al. (1998). Cell 93, 1195-1205
13. Lending and Larkins (1989). Plant Cell 1, 1011-1023
14. Hong et al. (1996). Development 122, 2051-2058.
15. Fernandez et al. (2000). Science 289, 436-438
16. D'Aoust et al. (1999). Plant Cell 11, 2407-2418
17. Bewley (1997). Plant Cell 9, 1055-1066
18. Heath et al. (1986). Planta 169, 304-312
19. Browse et al. (1986). Anal. Biochem. 152, 141-145
20. D'Aoust et al. (1999). Plant Cell 11, 2407-2418

Other biological activities that can be modulated by the fruit-specific developmentally responsive polynucleotides and polynucleotide products are listed in Reference Tables. Assays for detecting such biological activities are described in the table as well as in the Protein Domain tables.

	BIOLOGICAL FUNCTION	POTENTIAL UTILITY	CITATION	ASSAY	CITATION
<b>Ovule Growth, Ovule</b>	Ethylene and ethylene signal	Manipulate female fertility.	De Martinis and Mariani (1999). Plant	Analyze ovule and seed	Winkler et al. (1998). Plant Physiol. 118,



<b>Development and Seed Growth and Development</b>	transduction pathway	Manipulate megasporogenesis.	Cell 11, 1061-1072. Silencing gene	development by light microscopy	743-750. Systematic reverse
	Examples: AP2 domain DNA binding proteins; EREBP, EBF Example: Leucine-rich receptor kinase; ETR-like Example: Raf kinase; CTR	Manipulate female gametophyte development. Manipulate fertilization independent endosperm development. Manipulate fertilization independent embryo development. Manipulate fertilization independent seed development. Manipulate ovule size. Manipulate endosperm size. Manipulate	expression of the ethylene-forming enzyme results in a reversible inhibition of ovule development in transgenic tobacco plants. Christensen et al. (1997). Sexual Plant Reproduc. 10, 49-64. Megagametogenesis in Arabidopsis wild type and the Gf mutant. Christiansen and Drews, unpublished	or by confocal microscopy. Test for fertilization independent endosperm development. Test for fertilization independent embryo development. Test for fertilization independent seed production. Analyze seed size. Analyze seed yield. Analyze seed composition. Analyze fruit size.	genetics of transfer-DNA-tagged lines of Arabidopsis. Weigel et al. (2000). Plant Physiol 122, 1003-1014. Activation tagging in Arabidopsis. Ohad et al. (1996). PNAS USA 93, 5319-5324. A mutation that allows endosperm development without fertilization Chaudhury et al. (1997). PNAS USA 94, 4223-4228. Fertilization-

		embryo size.  Manipulate seed size.  Manipulate seed yield.  Manipulate seed protein.  Manipulate seed oil.  Manipulate starch production.  Manipulate cell number.  Manipulate cell size.  Produce seedless fruit and vegetables  Manipulate fruit size.			independent seed development in Arabidopsis thaliana   De Martinis and Mariani (1999). Plant Cell 11, 1061- 1072. Silencing gene expression of the ethylene- forming enzyme results in a reversible inhibition of ovule development in transgenic tobacco plants.  Christensen et al. (1997). Sexual Plant Reproduc. 10, 49-64.  Megagametoge- nesis in Arabidopsis
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					<p>wild type and the Gf mutant.</p> <p>Scott et al. (1998). Development 125, 3329-3341. Parent-of-origin effects on seed development in Arabidopsis thaliana</p> <p>Heath et al. (1986). Planta 169, 304-312.</p> <p>Browse et al. (1986). Anal. Biochem. 152, 141-145.</p> <p>D'Aoust et al. (1999). Plant Cell 11, 2407-2418.</p>
	<b>2. Growth and developmental control</b>	Manipulate female fertility.	Wilson et al. (1996). Plant Cell 8, 659-671. A	Analyze ovule and seed development by light	Winkler et al. (1998). Plant Physiol. 118, 743-750.

<i>genes</i> ---- <i>Upregulated genes</i> Example: DNA binding proteins; tiny-like, AGL1, FBP2, AGL9, AP3, CPC-like myb.  Example: Protein kinase; ASK1. Example: Auxin conjugating enzyme; indole-3-acetate beta-glucosyltransferase.  Example: S/T protein kinase; APK1. Example:	Manipulate megasporogenesis.  Manipulate female gametophyte development.  Manipulate fertilization independent endosperm development.  Manipulate fertilization independent embryo development.  Manipulate fertilization independent seed development.  Manipulate ovule size.  Manipulate endosperm size.	dissociation insertion causes a semidominant mutation that increases expression of TINY, an Arabidopsis gene related to APETALA2.  Zhao et al (1999). Developmental Genetics 25, 209-223. The ASK1 gene regulates development and interacts with the UFO gene to control floral organ identity in Arabidopsis. Flanagan et al. (1996). Plant J. 10, 343-53.	microscopy or by confocal microscopy.  Test for fertilization independent endosperm development.  Test for fertilization independent embryo development.  Test for fertilization independent seed production.  Analyze seed size.  Analyze seed yield.  Analyze seed composition.  Analyze fruit size.  Analyze seedling size.  Analyze	Systematic reverse genetics of transfer-DNA-tagged lines of Arabidopsis. Weigel et al. (2000). Plant Physiol 122, 1003-1014. Activation tagging in Arabidopsis. Ohad et al. (1996). PNAS USA 93, 5319-5324. A mutation that allows endosperm development without fertilization Chaudhury et al. (1997). PNAS USA 94, 4223-4228. Fertilization-
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	<p>Leucine-rich receptor kinase; CLV1, ER, BRI, Cf-2-like.</p> <p>-----</p> <p><i>Downregulate d genes</i></p> <p>Example: Cyclin-dependent kinase; cdc2.</p>	<p>Manipulate embryo size.</p> <p>Manipulate organ size and number.</p> <p>Manipulate seed size.</p> <p>Manipulate seed yield.</p> <p>Manipulate seedling size through seed size.</p> <p>Manipulate seedling vigor through seed size.</p> <p>Manipulate seed protein.</p> <p>Manipulate seed oil.</p> <p>Manipulate starch production.</p> <p>Manipulate integument development.</p> <p>Manipulate</p>	<p>Specific expression of the AGL1 MADS-box gene suggests regulatory functions in Arabidopsis gynoecium and ovule development.</p> <p>Angenent et al. (1994). Plant J 1994. 5, 33-44. Co-suppression of the petunia homeotic gene fbp2 affects the identity of the generative meristem.</p> <p>AGL9 web page.</p> <p>Wada et al. (1997) Science 277, 1113-6. Epidermal cell</p>	<p>seedling viability.</p> <p>Screen for changes in shatter time.</p> <p>Screen for changes in germination frequency.</p> <p>Screen for seed longevity and viability.</p>	<p>independent seed development in Arabidopsis thaliana</p> <p>De Martinis and Mariani (1999). Plant Cell 11, 1061-1072. Silencing gene expression of the ethylene-forming enzyme results in a reversible inhibition of ovule development in transgenic tobacco plants.</p> <p>Christensen et al. (1997). Sexual Plant Reproduc. 10, 49-64. Megagametog</p>
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		seedcoat development. Manipulate cell size. Manipulate cell number. Manipulate homeotic gene expression. Manipulate organ size. Manipulate meristem size. Produce seedless fruit and vegetables Manipulate fruit size. Manipulate time of seed dispersal. Manipulate seed viability upon storage. Manipulate germination	differentiation in Arabidopsis determined by a Myb homolog CPC. Szerszen et al. (1994). Science 16, 1699-1701. iaglu, a gene from Zea mays involved in conjugation of growth hormone indole-3- acetic acid. Ito et al. (1997). Plant Cell Physiol. 38, 248-258. A serine/threoni ne protein kinase gene isolated by an in vivo binding		nesis in Arabidopsis wild type and the Gf mutant. Scott et al. (1998). Development 125, 3329- 3341. Parent- of-origin effects on seed development in Arabidopsis thaliana. Heath et al. (1986). Planta 169, 304-312. Browse et al. (1986). Anal. Biochem. 152, 141-145. D'Aoust et al. (1999). Plant Cell 11, 2407- 2418.
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		frequency.	<p>procedure using the Arabidopsis floral homeotic gene product, AGAMOUS.</p> <p>Clark et al. (1997). Cell 89, 575-585.</p> <p>The CLAVATA1 gene encodes a putative receptor kinase that controls shoot and floral meristem size in Arabidopsis.</p> <p>Torii et al. (1996). Plant Cell 8, 735- 746. The Arabidopsis ERECTA gene encodes a putative receptor</p>		
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			protein kinase with extracellular leucine-rich repeats.  Li and Chory (1997). Cell 90, 929-38. A putative leucine-rich repeat receptor kinase involved in brassinosteroid signal transduction.		
	<b>3. Cell senescence and cell death</b>  Example: Cystatin Example: WIPK	Manipulate female fertility.  Manipulate seed set.  Manipulate seed yield.  Manipulate seed size.  Manipulate fruit set.  Promote	Solomon et al. (1999). Plant Cell 11, 431-444. The involvement of cysteine proteases and protease inhibitor genes in the regulation of programmed cell death in	Analyze ovule and seed development by light microscopy or by confocal microscopy.  Analyze seed set.  Analyze seed size.  Analyze seed	Winkler et al. (1998). Plant Physiol. 118, 743-750. Systematic reverse genetics of transfer-DNA-tagged lines of Arabidopsis. Weigel et al. (2000). Plant



		apomixis.  Produce seedless fruit and vegetables.	plants.  Zhang et al. (2000). Plant J. 23, 339-347.  Multiple levels of tobacco WIPK activation during the induction of cell death by fungal elicitors.	yield.  Analyze fruit set.  Screen for fertilization independent seed development.	Physiol 122, 1003-1014.  Activation tagging in Arabidopsis.  Ohad et al. (1996). PNAS USA 93, 5319-5324. A mutation that allows endosperm development without fertilization
	<b>4. Protein remodelin g</b>  Example: DNA-J protein/chaperones	Manipulate female fertility.  Manipulate female gametophyte development.  Promote apomixis.  Manipulate endosperm development.  Manipulate	Christensen et al. (1997). Sexual Plant Reproduc. 10, 49-64.  Megagametogenesis in Arabidopsis wild type and the Gf mutant.  Cory Christiansen and Gary Drews,	Test for altered female fertility, seed set, seed yield.  Analyze ovule development by light microscopy or by confocal microscopy.  Analyze seed size.	Winkler et al. (1998). Plant Physiol. 118, 743-750.  Systematic reverse genetics of transfer-DNA-tagged lines of Arabidopsis.  Weigel et al. (2000). Plant Physiol 122,

		embryo development. Manipulate seed size. Manipulate seed yield. Manipulate seed protein. Manipulate seed oil. Manipulate starch. Produce seedless fruit and vegetables.	unpublished	Analyze seed yield. Analyze seed composition.	1003-1014. Activation tagging in Arabidopsis. Christensen et al. (1997). Sexual Plant Reproduc. 10, 49-64. Megagametogenesis in Arabidopsis wild type and the Gf mutant. Ohad et al. (1996). PNAS USA 93, 5319-5324. A mutation that allows endosperm development without fertilization Scott et al. (1998). Development 125, 3329-3341. Parent-of-origin
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					effects on seed development in Arabidopsis thaliana.  Heath et al. (1986). Planta 169, 304-312.  Browse et al. (1986). Anal. Biochem. 152, 141-145.  D'Aoust et al. (1999). Plant Cell 11, 2407-2418.
	5. <i>Sucrose mobilization and partitioning</i>  Example: Invertase inhibitor  Example: bZIP transcription factor (translation of	Manipulate female fertility.  Manipulate ovule development.  Manipulate seed development.  Manipulate endosperm	Mapping of tomato genes associated with sugar metabolism.  Tomato Genetics Co-op Report 48, 22-23 (1998)  Ikeda et al. (1999). Plant Physiol 121,	Analyze ovule and seed development by light microscopy or by confocal microscopy.  Determine female fertility.  Analyze seed	Winkler et al. (1998). Plant Physiol. 118, 743-750.  Systematic reverse genetics of transfer-DNA-tagged lines of Arabidopsis.  Weigel et al. (2000). Plant

	<p>bZIP protein is inhibited by sucrose levels greater than 25 mM)</p> <p>Example: Lipoxygenase</p> <p>----</p> <p><i>Downregulate d gene</i></p> <p>Example: SNF1-related protein kinase</p>	<p>development.</p> <p>Manipulate embryo development.</p> <p>Manipulate seed size.</p> <p>Manipulate seed yield.</p> <p>Manipulate seed protein.</p> <p>Manipulate seed oil.</p> <p>Manipulate starch.</p> <p>Manipulate cell size.</p> <p>Manipulate cell number.</p> <p>Manipulate organ size.</p> <p>Manipulate meristem size.</p> <p>Manipulate seedling size through seed size.</p> <p>Manipulate</p>	<p>813-820.</p> <p>Sucrose and Cytokinin Modulation of WPK4, a Gene Encoding a SNF1-Related Protein Kinase from Wheat.</p> <p>Rook et al. (1998). Plant J. 15, 253-263. Sucrose-specific signaling represses translation of the Arabidopsis ATB2 bZIP transcription factor gene.</p> <p>Rook et al. (1998). Plant Mol Biol 37,171-178. The light-regulated</p>	<p>mass.</p> <p>Analyze seed yield.</p> <p>Analyze seed composition.</p> <p>Analyze organ size.</p> <p>Analyze seedling size.</p> <p>Analyze seedling viability.</p>	<p>Physiol 122, 1003-1014. Activation tagging in Arabidopsis. Christensen et al. (1997). Sexual Plant Reproduc. 10, 49-64. Megagametogenesis in Arabidopsis wild type and the Gf mutant. Ohad et al. (1996). PNAS USA 93, 5319-5324. A mutation that allows endosperm development without fertilization</p> <p>Scott et al. (1998). Development 125, 3329-</p>
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		seedling viability through seed size. Produce seedless fruit and vegetables. Translational control of gene expression in ovule and seed by sucrose. Manipulate assimilate partitioning in ovule and seed development.	Arabidopsis bZIP transcription factor gene ATB2 encodes a protein with an unusually long leucine zipper domain. Bunker et al. (1995). Plant Cell 7, 1319-1331. Sink limitation induces the expression of multiple soybean vegetative lipoxxygenase mRNAs while the endogenous jasmonic acid level remains low. Lowry et al. (1998). Plant		3341. Parent-of-origin effects on seed development in Arabidopsis thaliana. 6. Heath et al. (1986). Planta 169, 304-312. 7. Browse et al. (1986). Anal. Biochem. 152, 141-145. 8. D'Aoust et al. (1999). Plant Cell 11, 2407-2418.
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			Physiol. 116, 923-933. Specific soybean lipoxygenases localize to discrete subcellular compartments and their mRNAs are differentially regulated by source-sink status.		
	<p><b>6. <i>Jasmonic acid biosynthesis and signal transduction pathway</i></b></p> <p>Example: Biosynthetic enzyme; FMN oxidoreductase 12-</p>	<p>Targeted death of cells belonging to the female gametophyte, ovule or integuments.</p> <p>Delay senescence of unfertilized female gametophyte, ovule or integuments.</p>	<p>Sanders et al. (2000). Plant Cell 12, 1041-1062. The Arabidopsis DELAYED DEHISCENCE1 gene encodes an enzyme in the jasmonic acid synthesis pathway.</p> <p>Vijayan et al.</p>	<p>Test for altered female fertility.</p> <p>Analyze male fertility.</p> <p>Screen for enhanced expression of pathogen defense response genes.</p>	<p>Winkler et al. (1998). Plant Physiol. 118, 743-750. Systematic reverse genetics of transfer-DNA-tagged lines of Arabidopsis</p> <p>Weigel et al. (2000). Plant Physiol 122,</p>

	oxophyto- dienoate reductase, OPR1, OPR1- like.  Example: Signal transduction pathway kinase WIPK.	Manipulate female fertility  Coordinate female with male reproduction.  Manipulate male fertility.  Enhanced defense response in ovules and seed	(1998). A role for jasmonate in pathogen defense of Arabidopsis. PNAS USA 95, 7209- 7214.  Seo et al. (1999). Plant Cell 11, 289- 298.  Jasmonate- based wound signal transduction requires activation of WIPK, a tobacco mitogen- activated protein kinase.		1003-1014.  Activation tagging in Arabidopsis.
<i>Environment al responses</i>	<i>1. Wound and defense response gene</i>	Pathogen resistant ovules.  Pathogen	Song et al. (1995). Science 270, 1804-1806. A	Resistance to Xanthamonas sp.  Resistance to	Winkler et al. (1998). Plant Physiol. 118, 743-750.

	<p><b><i>expression</i></b></p> <p>Example: Leucine rich receptor S/T kinase; Xa21- like and TMK-like.</p> <p>Example: Cell wall- associated protein kinase WAK1.</p> <p>Example: Thionins.</p>	<p>resistant seeds.</p> <p>Pathogen resistant fruit.</p>	<p>receptor kinase-like protein encoded by the rice disease resistance gene, Xa21.</p> <p>Seo et al. (1995). Science 270, 1988- 1992. Tobacco MAP kinase: a possible mediator in wound signal transduction pathways.</p> <p>He et al. (1998). Plant J. 14, 55-63. Requirement for the induced expression of a cell wall associated receptor kinase for survival during the pathogen response.</p>	<p>known arabidopsis pathogens in ovules, seed and fruit.</p>	<p>Systematic reverse genetics of transfer-DNA- tagged lines of Arabidopsis.</p> <p>Weigel et al. (2000). Plant Physiol 122, 1003-1014. Activation tagging in Arabidopsis.</p> <p>Epple and Bohlmann (1997). Plant Cell 9, 509- 520. Overexpress- ion of an endogenous thionin enhances resistance of Arabidopsis against Fusarium oxysporum. He et al.</p>
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			<p>He et al. (1999). Plant Mol. Biol. 39, 1189-1196. A cluster of five cell wall- associated receptor kinase genes, Wak1-5, are expressed in specific organs of Arabidopsis.</p> <p>Epple and Bohlmann (1997). Plant Cell 9, 509- 520. Overexpressi on of an endogenous thionin enhances resistance of Arabidopsis against Fusarium oxysporum.</p> <p>Ichimura et al.</p>		<p>(1998). Plant J. 14, 55-63. Requirement for the induced expression of a cell wall associated receptor kinase for survival during the pathogen response.</p>
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			(1998). DNA Res. 5,341-5348. Molecular cloning and characterization of three cDNAs encoding putative mitogen-activated protein kinase kinases (MAPKKs) in <i>Arabidopsis thaliana</i> .		
	<p><b>2. <i>Stress response to cold, drought, salinity, seed maturation, embryo development, ABA.</i></b></p> <p>Example: Dehydrins</p> <p>Example: NPK1-like protein kinase</p>	<p>Manipulate drought resistance.</p> <p>Manipulate desiccation tolerance in flowers, ovules and seeds.</p> <p>Manipulate cold tolerance in flowers, ovules, and seeds.</p>	<p>Close, T.J. (1996). <i>Physiol.Plant</i> 97, 795-803.</p> <p>Dehydrins: emergence of a biochemical role of a family of plant dehydration proteins.</p> <p>Kovtun et al. (2000). <i>PNAS</i></p>	<p>Test for enhanced sensitivity to drought, dessication, cold, salinity, in ovules, developing seed and seedlings.</p> <p>Test for enhanced tolerance to drought,</p>	<p>Winkler et al. (1998). <i>Plant Physiol.</i> 118, 743-750.</p> <p>Systematic reverse genetics of transfer-DNA-tagged lines of <i>Arabidopsis</i>.</p> <p>Weigel et al. (2000). <i>Plant Physiol</i> 122,</p>

	Example: DNA binding protein genes: CBF-like, DREB2A, RAP2.1.	Manipulate seed dormancy.  Manipulate germination frequency.  Manipulate seed storage and viability.	USA 97, 2940-2945.  Functional analysis of oxidative stress- activated mitogen- activated protein kinase cascade in plants.	dessication, cold, salinity, in ovules, developing seed and seed.  Test for changes in seed viability upon storage.  Test for changes in germination frequencies.	1003-1014.  Activation tagging in Arabidopsis.
	<b>3. Response to starvation, wounding, and pathogen attack by tryptophan synthesis.</b>  Example: DNA binding protein; ATR1-like myb.  Example: Auxin conjugating enzyme; indole-3-	Altered response to starvation.  Altered response to wounding.  Altered response to pathogen attack.	Bender and Fink (1998).  A myb homologue, ATR1, activates tryptophan gene expression in arabidopsis. PNAS USA 95, 5655- 5660.	Test for enhanced sensitivity to starvation, wounding, and pathogen attack.  Test for enhanced tolerance to starvation, wounding, and pathogen attack.	Winkler et al. (1998). Plant Physiol. 118, 743-750.  Systematic reverse genetics of transfer- DNA-tagged lines of Arabidopsis.  Weigel et al. (2000). Plant Physiol 122, 1003-1014. Activation tagging in Arabidopsis.

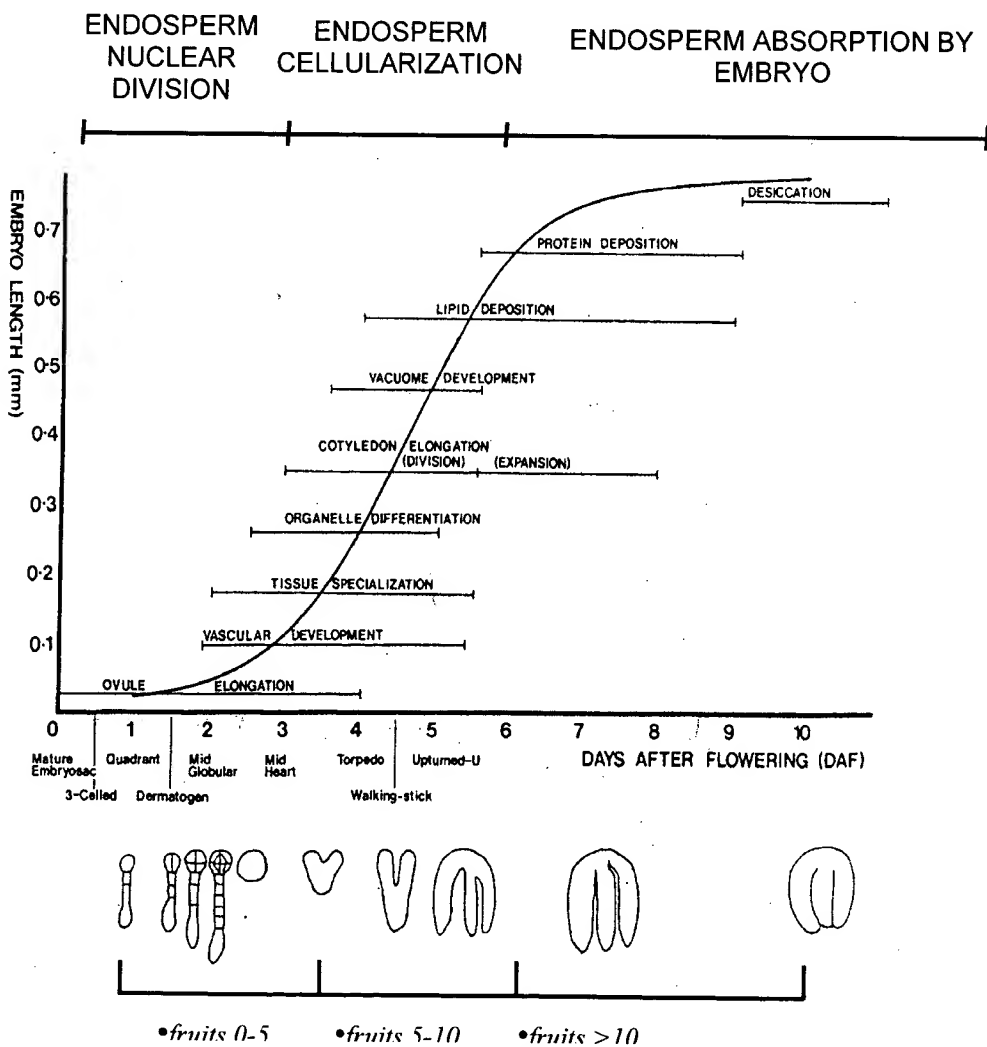
	acetate beta-glucosyltransferase.				
<b>Cell metabolism</b>	<b><i>Stearoyl-acyl carrier protein desaturase</i></b>  Example: C18 fatty acid desaturation	Production of oils high in saturated fatty acids  Manipulate membrane composition	Merlo et al. (1998). Plant Cell 10, 1603-1621.	Analyze seed size.  Analyze seed yield.  Analyze seed composition.  Analyze seed oil by gas chromatography.	Winkler et al. (1998). Plant Physiol. 118, 743-750.  Systematic reverse genetics of transfer-DNA-tagged lines of Arabidopsis.  Weigel et al. (2000). Plant Physiol 122, 1003-1014.  Activation tagging in Arabidopsis.  Browse et al. (1986). Anal. Biochem. 152, 141-145.
	<b>2. Manipulate</b>	Manipulate asparagine	Mathews and Van Holde	Analyze seed size.	Winkler et al. (1998). Plant

	<p><b><i>nitrogen economy</i></b></p> <p>Example: Asparaginase</p>	<p>degradation in ovules and seeds.</p> <p>Manipulate endosperm production.</p> <p>Manipulate embryo development.</p> <p>Manipulate ovule size.</p> <p>Manipulate seed size.</p>		<p>Analyze seed yield.</p> <p>Analyze seed composition.</p>	<p>Physiol. 118, 743-750.</p> <p>Systematic reverse genetics of transfer-DNA-tagged lines of Arabidopsis.</p> <p>Weigel et al. (2000). Plant Physiol 122, 1003-1014.</p> <p>Activation tagging in Arabidopsis.</p> <p>Heath et al. (1986). Planta 169, 304-312.</p> <p>Browse et al. (1986). Anal. Biochem. 152, 141-145.</p> <p>D'Aoust et al. (1999). Plant Cell 11, 2407-2418.</p>
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Fruit development responsive polynucleotides are characteristically differentially

transcribed in response to fluctuating developmental-specific polynucleotide levels or other signals, whether internal or external to a cell. MA\_diff and/or AFLP\_diff and/or AFLP\_diff reports the changes in transcript levels of various fruit development responsive polynucleotides in fruits.

5 These data can be used to identify a number of types of fruit development responsive polynucleotides. Profiles of some of these different fruit development responsive polynucleotides are shown in the table below with examples of the kinds of associated biological activities. Because development is a continuous process and many cell types are being examined together, the expression profiles of genes overlap between stages of development in the chart below.



Transcript Levels	Developmental Process	Metabolic Pathways	Examples of Biochemical Activity
<p>(0-5 mm)&gt;&gt;(5-10 mm) <math>\cong</math> (&gt;10 mm)</p> <p>(0-5 mm)&gt;&gt;(5-10 mm) &gt; (&gt;10 mm)</p> <p>(0-5 mm)&gt;(5-10 mm) <math>\cong</math> (&gt;10 mm)</p>	<p>Ovule Elongation</p> <p>Tissue Specialization</p> <ul style="list-style-type: none"> <li>- Vascular system</li> <li>- Meristem</li> <li>- Endosperm</li> <li>- Seed coat</li> <li>- Fruit</li> </ul>	<ul style="list-style-type: none"> <li>- Hormone Production, Transport, Perception, Signalling, Response (e.g., Gibberellin, Ethylene, Auxin)</li> <li>- Cell wall Biosynthesis</li> <li>- Lipid Biosynthesis</li> <li>- Specific Gene Transcription Initiation</li> <li>- Sucrose Mobilization and Partitioning</li> <li>- Sucrose Signaling</li> <li>- Lipoxygenase Localization</li> <li>- Repressors of Metabolic Pathways</li> <li>- Protein Remodeling</li> </ul>	<ul style="list-style-type: none"> <li>- Transcription Factors</li> <li>- Transporters</li> <li>- Kinases</li> <li>- Changes in cytoskeletal protein activity modulating cell structure</li> <li>- Stability factors for protein translation</li> <li>- Changes in cell wall/membrane structure</li> <li>- Chromatin structure and/or DNA topology</li> <li>- Biosynthetic enzymes</li> </ul>
<p>(5-10 mm) &gt;&gt;(0-5 mm) &gt; (&gt;10 mm)</p> <p>(5-10 mm) &gt;(0-5 mm) <math>\cong</math> (&gt;10 mm)</p> <p>(5-10 mm) &gt;&gt;(0-5 mm) <math>\cong</math> (&gt;10 mm)</p>	<p>Tissue Specialization</p> <ul style="list-style-type: none"> <li>- Vascular System</li> </ul> <p>Organelle Differentiation</p> <p>Cotyledon Elongation (cell division)</p> <p>Vacuome Development</p> <p>Lipid Deposition</p>	<ul style="list-style-type: none"> <li>- Cell Wall Biosynthesis</li> <li>- Specific Gene Transcription Initiation</li> <li>- Sucrose Mobilization and Partitioning</li> <li>- Sucrose Signaling</li> <li>- Repressors of Metabolic Pathways</li> <li>- Auxin Perception, Response and Signaling</li> <li>- Protein Remodeling</li> <li>- Lipid Biosynthesis and Storage</li> </ul>	<ul style="list-style-type: none"> <li>- Transcription Factors</li> <li>- Transporters</li> <li>- Kinases</li> <li>- Chaperones</li> <li>- Changes in cytoskeletal protein activity modulating cell structure</li> <li>- Stability of factors for protein translation</li> <li>- Changes in cell wall/membrane structure</li> <li>- Chromatin structure and/or DNA topology</li> <li>- Biosynthetic enzymes</li> </ul>

Transcript Levels	Developmental Process	Metabolic Pathways	Examples of Biochemical Activity
(>10 mm) > (0-5 mm) $\cong$ (5-10 mm)	Cotyledon Elongation (expansion) Lipid Deposition Protein Deposition Desiccation	<ul style="list-style-type: none"> <li>- Cell Elongation</li> <li>- Specific Gene Transcription Initiation</li> <li>- Sucrose Mobilization and Partitioning</li> <li>- Sucrose Signaling</li> <li>- Lipoxygenase Localization</li> <li>- Repressors of metabolic pathways</li> <li>- Hormone Perception, Response and Signaling (e.g. abscissic acid)</li> <li>- Protein Remodeling</li> <li>- Protein synthesis and Storage</li> <li>- Lipid Synthesis and Storage</li> <li>- Acquisition of Dessication Tolerance</li> <li>- Senescence</li> </ul>	<ul style="list-style-type: none"> <li>- Transcription Factors</li> <li>- Transporters</li> <li>- Kinases</li> <li>- Chaperones for protein translation</li> <li>- Changes in cell wall/membrane structure</li> <li>- Chromatin structure and/or DNA topology</li> <li>- Biosynthetic enzymes</li> <li>- Metabolic enzymes</li> </ul>
(0-5 mm) < (5-10 mm) $\cong$ (>10 mm)  (0-5 mm) << (5-10 mm) $\cong$ (>10 mm)  (0-5 mm) << (5-10 mm) < (>10 mm)  (0-5 mm) << (>10 mm) < (5-10 mm)	Ovule Elongation -Repressors of Ethylene production Tissue specialization - Vascular System - Meristem - Cotyledon - Seed Coat	<ul style="list-style-type: none"> <li>- Cell elongation</li> <li>- Negative regulation of ethylene pathways</li> <li>- Maintenance of Ethylene response</li> <li>- Changes in pathways and processes operation in cells</li> </ul>	<ul style="list-style-type: none"> <li>- Transcription Factors</li> <li>- Transporters</li> <li>- Kinases</li> <li>- Chaperones</li> <li>- Stability of factors</li> <li>- Biosynthetic enzymes</li> <li>- Metabolic enzymes</li> </ul>
(5-10 mm) < (0-5 mm) $\cong$ (>10 mm)	Organelle differentiation Cotyledon elongation (division) Vacuome development Lipid development Desiccation	<ul style="list-style-type: none"> <li>- Negative regulation of hormone pathways</li> <li>- Maintenance of hormone response</li> <li>- Changes in pathways and processes operation in cells</li> <li>- Dehydration and acquisition of desiccation tolerance</li> <li>- Senescence</li> </ul>	<ul style="list-style-type: none"> <li>- Transcription Factors</li> <li>- Transporters</li> <li>- Kinases</li> <li>- Chaperones</li> </ul>
(>10 mm) < (0-5 mm)	Cotyledon Elongation	- Cell elongation	- Transcription



Transcript Levels	Developmental Process	Metabolic Pathways	Examples of Biochemical Activity
mm) $\cong$ (5-10 mm)	(expansion) Lipid deposition Protein deposition Desiccation	<ul style="list-style-type: none"> <li>- Negative regulation of hormone pathways</li> <li>- Maintenance of hormone response</li> <li>- Changes in pathways and processes operation in cells</li> <li>- Dehydration and acquisition of desiccation tolerance</li> <li>- Senescence</li> </ul>	<p>Factors</p> <ul style="list-style-type: none"> <li>- Transporters</li> <li>- Kinases</li> <li>- Chaperones</li> <li>- Metabolic enzymes</li> <li>- Biosynthetic enzymes</li> </ul>
(0-5 mm) $\cong$ (5-10 mm) $\cong$ (>10 mm)	All stages	<ul style="list-style-type: none"> <li>- Ribosome/polysome production and maintenance</li> <li>- Housekeeping genes</li> </ul>	<ul style="list-style-type: none"> <li>- Transcription Factors</li> <li>- Transporters</li> <li>- Kinases</li> <li>- Chaperones</li> </ul>

I.D. DEVELOPMENT GENES, GENE COMPONENTS AND PRODUCTS

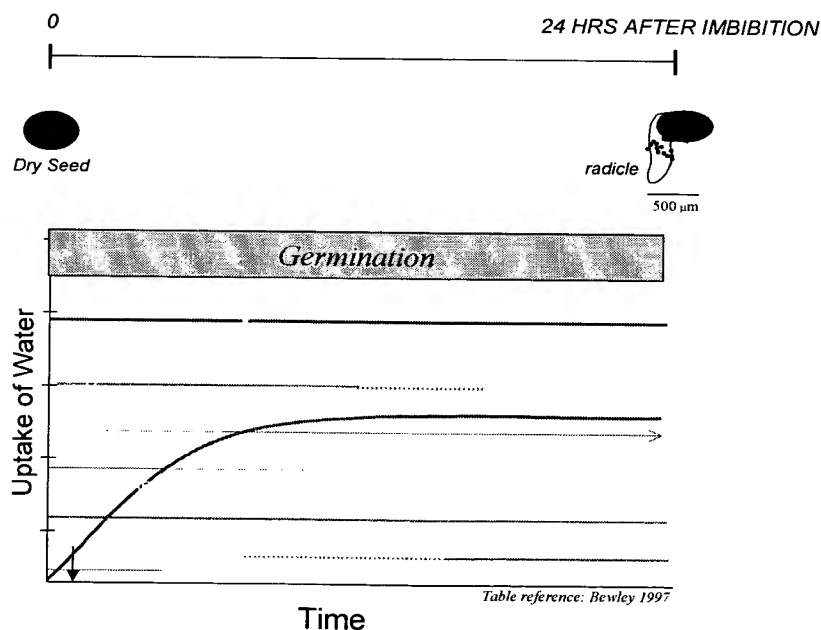
I.D.1. IMBIBITION AND GERMINATION RESPONSIVE GENES, GENE  
COMPONENTS AND PRODUCTS

Seeds are a vital component of the world's diet. Cereal grains alone, which comprise ~90% of all cultivated seeds, contribute up to half of the global per capita energy intake. The primary organ system for seed production in flowering plants is the ovule. At maturity, the ovule consists of a haploid female gametophyte or embryo sac surrounded by several layers of maternal tissue including the nucleus and the integuments. The embryo sac typically contains seven cells including the egg cell, two synergids, a large central cell containing two polar nuclei, and three antipodal cells. that . Pollination results in the fertilization of both egg and central cell. The fertilized egg develops into the embryo. The fertilized central cell develops into the endosperm. And the integuments mature into the seed coat. As the ovule develops into the seed, the ovary matures into the fruit or silique. Late in development, the developing seed ends a period of extensive biosynthetic and cellular activity and begins to desiccate to complete its development and enter a dormant, metabolically quiescent state. Seed dormancy is generally an undesirable characteristic in agricultural crops, where rapid germination and growth are required. However, some degree of dormancy is advantageous, at least during seed development. This is particularly true for cereal crops because it prevents germination of grains while still on the ear of the parent plant (preharvest sprouting), a phenomenon that results in major losses to the agricultural industry. Extensive domestication and breeding of crop species have ostensibly reduced the level of dormancy mechanisms present in the seeds of their wild ancestors, although under some adverse environmental conditions, dormancy may reappear. By contrast, weed seeds frequently mature with inherent dormancy mechanisms that allow some seeds to persist in the soil for many years before completing germination.

Germination commences with imbibition, the uptake of water by the dry seed, and the activation of the quiescent embryo and endosperm. The result is a burst of intense metabolic activity. At the cellular level, the genome is transformed from an inactive state to one of intense transcriptional activity. Stored lipids, carbohydrates and proteins are catabolized fueling seedling

growth and development. DNA and organelles are repaired, replicated and begin functioning. Cell expansion and cell division are triggered. The shoot and root apical meristem are activated and begin growth and organogenesis. Schematic 4 summarizes some of the metabolic and cellular processes that occur during imbibition. Germination is complete when a part of the embryo, the radicle, extends to penetrate the structures that surround it. In Arabidopsis, seed germination takes place within twenty-four (24) hours after imbibition. As such, germination requires the rapid and orchestrated transcription of numerous polynucleotides. Germination is followed by expansion of the hypocotyl and opening of the cotyledons. Meristem development continues to promote root growth and shoot growth, which is followed by early leaf formation.

*Schematic 4. Cellular and Molecular Events in Seed Imbibition and Germination*



Genes with activities relevant to imbibition-germination and early seedling growth are described in the two sections A and B below.

#### D.2.a. IDENTIFICATION OF IMBIBITION AND GERMINATION GENES

5 Imbibition and germination includes those events that commence with the uptake of water by the quiescent dry seed and terminate with the expansion and elongation of the shoots and roots. The germination period exists from imbibition to when part of the embryo, usually the radicle, extends to penetrate the seed coat that surrounds it.

10 Imbibition and germination genes identified herein are defined as genes, gene components and products capable of modulating one or more processes of imbibition and germination described above. They are useful to modulate many plant traits from early vigor to yield to stress tolerance. Examples of such germination genes and gene products are shown in the Reference and Sequence Tables. The functions of many of the genes were deduced from comparisons with known proteins and are also given in the REF Tables.

#### Imbibition and Germination Genes Identified by Phenotypic Observations

15 Imbibition and germination genes are active, potentially active or more active during growth and development of a dry seed into a seedling. These genes herein were discovered and characterized from a much larger set of genes in experiments designed to find genes that cause poor germination.

20 In these experiments, imbibition and germination genes were identified by either 1) ectopic expression of a cDNA in a plant or (2) mutagenesis of the plant genome. The seeds were then imbibed and cultivated under standardized conditions and any phenotypic differences in the modified plants compared with the parental "wild-type" seedlings were recorded. The genes causing the changes were deduced from the cDNA inserted or gene mutated. The phenotypic differences observed were poor germination and aberrant seedlings.

#### Imbibition and Germination Genes Identified by Differential Expression

25 Germination genes were also identified by measuring the relative levels of mRNA products of genes in different stages of germination of a seed versus the plant as a whole. Specifically, mRNA was isolated from whole imbibed seeds of Arabidopsis plants 1, 2, 3 or 4

days after imbibition and compared to mRNA isolated from dry seed-utilizing microarray procedures. The MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) report the transcript levels of the experiment. For transcript levels that were higher in the imbibed seed than in dry seed a "+" is shown. A "-" is shown when the transcript levels in dry seed were greater than those in imbibed seed. For more experimental detail, see the examples below:

Germination associated genes can be identified by comparing expression profiles of imbibed gibberellin treated and untreated gal mutant seed. Germination associated genes can also be identified by comparing expression profiles in late maturation seed from wild-type and mutants that are defective for the establishment of dormancy and can germinate precociously (e.g. aba1, aba2, abi4 in arabidopsis and vp1, vp5 in maize) or are defective for the specification of cotyledon identity and dessication tolerance (e.g. lec1, lec2, and fus3).

The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108461, 108462, 108463, 108464, 108528, 108529, 108530, 108531, 108545, 108546, 108547, 108518, 108529, 108543, 108544). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Imbibed & Germinating Seeds genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### Imbibed & Germinating Seeds Genes Identified By Cluster Analyses Of Differential Expression

#### Imbibed & Germinating Seeds Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Imbibed & Germinating Seeds genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108461, 108462, 108463, 108464, 108528, 108529, 108530, 108531, 108545, 108546, 108547, 108518, 108529, 108543, 108544 of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

Imbibed & Germinating Seeds Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Imbibed & Germinating Seeds genes. A group in the MA\_clust is considered a Imbibed & Germinating Seeds pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

Imbibed & Germinating Seeds Genes Identified By Amino Acid Sequence Similarity

Imbibed & Germinating Seeds genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Imbibed & Germinating Seeds genes. Groups of Imbibed & Germinating Seeds genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Imbibed & Germinating Seeds pathway or network is a group of proteins that also exhibits Imbibed & Germinating Seeds functions/utilities.

D.2.b. USE OF IMBIBITION AND GERMINATION GENES, GENE COMPONENTS  
AND PRODUCTS TO MODULATE PHENOTYPES

Imbibition and germination genes and gene products can be divided into those that act  
5 during primary events, secondary events, and/or termination. The genes and gene products of  
the instant invention are useful to modulate any one or more of the phenotypes described below:

I. Primary events

A. Dormancy

Imbibition and germination genes and gene products of the invention can act to modulate  
10 different types of dormancy including:

1. Primary dormancy – dormancy is established during seed development
2. Seed coat-imposed dormancy – dormancy is imposed by blocking water uptake, mechanical restraint of embryo, blocking the exit of inhibitors
3. Embryo dormancy – cotyledon mediated inhibition of embryonic axis growth
4. Secondary dormancy – dormancy is induced when dispersed, mature seeds are exposed to unfavorable conditions for germination (e.g. anoxia, unsuitable temperature or illumination).
5. Hormone-induced

B. Dormancy-breaking signal perception and transduction

Germination genes and gene products include those that are able to modulate the  
response to dormancy releasing signals such as:

1. Fruit ripening and seed development
2. Imbibition
3. Temperature – low and high (range 0-23°)
4. Light – particularly for coat imposed dormancy
  - a. White light
  - b. Intermittent illumination
  - c. Orange and red region of the spectrum (longer than 700 or 730 nm)

- d. Phytochrome
- 5. Coat softening
- 6. Chemicals
  - a. Respiratory inhibitors
  - b. Sulfhydryl compounds
  - c. Oxidants
  - d. Nitrogenous compounds
  - e. Growth regulators – GA, BA, ethylene
  - f. Various, ethanol, methylene blue, ethyl ether, fusicoccin
- 7. Oxygen and Carbon dioxide
- 8. Stress

## II. Secondary Events

During the secondary events of germination, dormancy-maintaining metabolism is repressed, dormancy-breaking metabolism is induced and structures surrounding the embryo weaken (where present). Germination genes and gene products are useful to modulate processes of the secondary events as follows:

- A. Water uptake
  - 1. Cell expansion
  - 2. Change in osmotic state
    - 1. Ion exchange
- B. Respiration – oxygen consumption

The genes and genes products of the invention can regulate the following pathways which resume during the first respiratory burst of germination:

- 1. Glycolysis
  - 2. Pentose phosphate
  - 3. Citric acid
  - 4. Tricarboxylic acid cycle
- C. Mitochondrial development



Tissues of the mature dry seed contain mitochondria, and although these organelles are poorly differentiated as a consequence of the drying, they contain sufficient Kreb's cycle enzymes and terminal oxidases to provide adequate amount of ATP to support metabolism for several hours after imbibition. During germination of embryos, there appears to be two distinct patterns of mitochondrial development. In starch-storing seeds, repair and activation of preexisting organelles predominate, whereas oil-storing seeds typically produce new mitochondria. Germination genes and gene products of the invention are useful to modulate the repair, activation and biogenesis pathways of mitochondria. Specific examples are as follows:

1. Membrane formation and repair
2. DNA repair and synthesis
3. Protein synthesis
4. Coordinated regulation of mitochondrial and nuclear genomes

#### D. Metabolism

In addition to respiration and organelle activity, enzyme activity, DNA repair, RNA synthesis and protein synthesis are fundamental cellular activities intimately involved in the completion of germination and the preparation for subsequent growth. Imbibition and germination genes and gene products of the invention can participate in or modulate these activities. Examples are:

1. ABA response(for more detail see section on ABA-responsive genes)
2. GA response(for more detail see section on GA-responsive genes)
3. ATP synthesis and Adenylate Energy Charge during germination
4. The synthesis and utilization of reducing power: pyridine nucleotides (NADH and NADPH)

### III. Termination

The last stage of seed germination is characterized by the emergence of the radicle or root apex through the seed coat. Typically, the cell walls loosen and the radicle extends from the embryo during late germination. Germination genes and gene products are useful to modulate

the mobilization of stored reserves, DNA synthesis and cell division that are typical of this stage of germination.

To regulate any of the phenotype(s) above, activities of one or more of the late germination genes or gene products can be modulated and tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266) and/or screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and visually inspected for the desired phenotype or metabolically and/or functionally assayed according to Dolan et al. (1993, Development 119: 71-84), Dolan et al. (1997, Development 124: 1789-98), Crawford and Glass (1998, Trends Plant Science 3: 389-95), Wang et al. (1998, PNAS USA 95: 15134-39), Gaxiola et al. (1998, PNAS USA 95: 4046-50), Apse et al. (1999, Science 285: 1256-58), Fisher and Long (1992, Nature 357: 655-60), Schneider et al. (1998, Genes Devel 12: 2013-21) and Hirsch (1999, Curr Opin Plant Biol. 2: 320-326).

#### D.2.c. USE OF IMBIBITION AND GERMINATION GENES, GENE COMPONENTS AND PRODUCT TO MODULATE BIOCHEMICAL ACTIVITIES

The roles of the biochemical changes associated with imbibition and germination can be appreciated from a summary of the processes occurring.

##### Physiology

Water plays an important role throughout the plant life cycle. The most dramatic example of this is in seed germination. Although germination is triggered by water, the germination response is also positively regulated by the plant growth regulators the gibberellins and negatively affected by the growth regulator abscisic acid. Genes that are activated by water and genes that are activated by gibberellins can be identified through expression profiling experiments using arabidopsis mutants defective for gibberellin biosynthesis or perception (gal, gai), abscisic acid biosynthesis or perception (aba1, abi3, and abi4) in the presence or absence of exogenous gibberellins. These genes can be used to promote seedling growth and development and other phases of plant development.

### Transcriptional Control of Gene Activity

At the end of seed development, dessication and dormancy have imposed a global state of repression on gene activity throughout the seed. Reactivation of the genome requires water and gibberellins. One function of the genes that are activated early by imbibition is the rapid and dramatic reversal of gene repression. For example, expression-profiling experiments revealed that several thousand genes are hyperactivated in arabidopsis upon imbibition. These include genes involved in metabolic pathways, genes that promote cell growth and division, and transcriptional control genes. Thus one class of genes expressed early in imbibition includes those that promote high levels of gene expression. Other early genes are responsible for regulating specific metabolic, cell, and developmental processes. The strategy for distinguishing these functions was outlined in the Introduction.

### Mobilization of Storage Reserves

In contrast to the synthesis and accumulation of reserves during seed development an important function of genes expressed during imbibition and germination is the control of the mobilization and catabolism of seed storage reserves in the endosperm (in grasses and cereals) and the embryo. The mobilization of seed storage reserves is triggered by imbibition and may occur over several days. There are three classes of high molecular weight seed storage reserves: carbohydrates, triacylglycerols, and storage proteins. Upon imbibition seed storage reserves are converted into forms that can be transported and metabolized. Genes encoding enzymes for storage reserve catabolism are expressed shortly after imbibition. Starch for example is converted to sucrose. Triacylglycerols are converted into acetyl-CoA. Storage proteins are converted into amino acids or deaminated to provide carbon skeletons for oxidation.

### Carbohydrate Catabolism

Starch is the most common storage carbohydrate in seeds. The primary components of starch are amylose and amylopectin.

### Mobilization

There are two pathways for starch catabolism – hydrolytic and phosphorolytic. The product of these pathways is the monosaccharide glucose. Examples of the enzymes responsible for hydrolytic catabolism of starch are: amylase, glucosidase, amylase, dextrinase, isoamylase. The enzyme responsible for phosphorolytic activity is starch phosphorylase.

5

#### Transport

The mobilization of starch involves the synthesis of sucrose from glucose, which can then be transported to sites for growth in the root and shoot. In some seeds, maltose may be a major form of transported carbohydrate. The production of sucrose-6-P from glucose involves the following enzymes: UDP-glucose pyrophosphorylase, sucrose-6-P synthetase, and sucrose phosphatase.

10

#### Sucrose Catabolism

In target tissues sucrose is hydrolyzed by fructofuransidase (invertase) and/or sucrose synthetase. The synthesis of glucose from glucose-1-P involves sucrose synthetase.

15

#### Cell Biology

The lumen of the endoplasmic reticulum (ER) is target for other hydrolase activities including mannosidase, glucosaminidase, acid phosphatase, phosphodiesterase, and phospholipase D.

20

### TRIACYLGLYCEROL (TAG) CATABOLISM

Triacylglycerols are the major storage lipids of seeds. The products of TAG catabolism in imbibed and germinating seed are glycerol and free fatty acids. Most of the glycerol is converted to sucrose for export. Free fatty acids are catabolized through oxidation through the glyoxylate cycle and gluconeogenesis.

25

#### Mobilization

Hydrolysis of triacylglycerols is by lipases yielding glycerol and free fatty acids. Free fatty acids are oxidized to acetyl-CoA and propionyl-CoA via oxidation requiring ATP and coenzyme A. Catabolism of unsaturated fatty acids also requires cis, trans-isomerases,

30

epimerases, and hydratases. Acetyl-CoA is oxidized through the citric acid cycle to CO<sub>2</sub> and H<sub>2</sub>O. More importantly, acetyl-CoA can be utilized via the glyoxylate cycle and gluconeogenesis for glucose synthesis. Free fatty acids are also broken down via oxidation. Glycerol is converted via phosphorylation and oxidation to DHAP and G3P, which are used to synthesize glucose or oxidized via the citric acid cycle. Examples of other induced enzymes include isocitrate lyase and malate synthetase

#### Transport

Most of the glycerol, acetyl-CoA, and propionyl-CoA are converted to sucrose for transport. This requires the enzymes glycerol kinase and glycerol phosphate oxidoreductase.

#### Cell Biology

Glyoxysome biogenesis is required to support fatty acid catabolism and gluconeogenesis. Upon exposure to light there is a loss of glyoxysomes due to their conversion to peroxisomes.

### STORAGE PROTEIN CATABOLISM

#### Mobilization

The hydrolysis of storage proteins to amino acids is performed by a diverse group of proteinases and peptidases. The peptidases include endopeptidases, aminopeptidases, and carboxypeptidases. They include the A and B class proteinases. The liberated amino acids are available for protein synthesis, for deamination and reutilization of ammonia via glutamine and asparagine synthesis, and to provide carbon skeletons for respiration. Several enzymes including, deaminase, asparagine synthetase, glutamine synthetase and glutamate dehydrogenase are important players in the mobilization and utilization of stored nitrogen in imbibed seed.

#### Transport

The major transported form of amino acid in germinated seeds is asparagine. In some species glutamine and/or homoserine are the major form of transported amino acid. Aspartate, glutamate, alanine, glycine, and serine can be converted to sucrose and transported as sucrose. Other amino acids are transported unchanged.

### Cell Biology

Proteinases are sequestered in lumen of endoplasmic reticulum (ER) which then fuses with protein bodies.

While catabolism is high in the storage tissues of imbibed seed the products of catabolism are transported to sites of growth including the shoot and root apices fueling respiration, biosynthesis, cell division and differentiation.

### DEVELOPMENT

Imbibition triggers several key processes for seedling development. One is the activation of the shoot and root apical meristems. The shoot apical meristem is responsible for two primary growth activities. One is the production of the protoderm, procambium and ground meristem. The protoderm gives rise to the epidermal system of the plant, the procambium to the primary vascular tissues, and the ground meristem to the ground tissues including the cortex and pith. The second is the production of leaf primordia, which arise on the flanks of the apex. Thus, activation of the shoot apical meristem results in shoot growth and organogenesis.

The root apical meristem, by contrast is responsible for vegetative root development. The first primary growth activity of the root apical meristem is the production of the protoderm, procambium and ground meristem. The second primary growth activity is the production of the cells that give rise to the root cap.

Genes that govern shoot apical meristem activation and development can be identified in arabidopsis by gene profiling experiments comparing gene expression in wild-type imbibed seed and partial loss-of-function stm (shootmeristemless ) mutants (see SAM). Genes governing root meristem activity can be identified by gene profiling experiments comparing gene expression in wild-type imbibed seed and rml (rootmeristemless) mutants.

Genes identified in this way are useful to promote or retard meristem growth, modify and strengthen shoot and root development, promote leaf development as described below.

Changes in the concentration of imbibition-germination activated polynucleotides result in the modulation of many other polynucleotides and polynucleotide products. Examples of such activated responsive polynucleotides and polynucleotide products relative to leaves and floral stem and to fruits at different development stages are shown in the Reference and Sequence Tables. These polynucleotides and/or products are responsible fore effects on traits such as

seedling growth, seedling viability, and seedling vigor. The polynucleotides were discovered by isolating seeds from Arabidopsis wild-type ecotype "Wassilewskija" imbibed for 24 hours, and measuring the mRNAs expressed in them relative to those in a leaf and floral stem sample and to those in fruits at different developmental stages.

While imbibition-germination activated polynucleotides and polynucleotide products can act alone, combinations of these polynucleotides also affect germination. Useful combinations include different polynucleotides and/or polynucleotide products that have similar transcription profiles or similar biological activities, and members of the same or functionally similar biochemical pathways. In addition, the combination of imbibition germination activated polynucleotides and/or polynucleotide products with environmentally responsive polynucleotides is also useful because of the interactions that exist between development, hormone-regulated pathways, stress and pathogen induced pathways and nutritional pathways. Here, useful combinations include polynucleotides that may have different transcription profiles, and participate in common or overlapping pathways but combine to produce a specific, phenotypic change.

Such imbibition and germination activated polynucleotides and polynucleotide products can function to either increase or dampen the above phenotypes or activities either in response to transcript changes in fruit development or in the absence of fruit-specific polynucleotide fluctuations.

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS ALTERED	CITATIONS INCLUDING ASSAYS
Growth , Differentiation and Development	Farnesylation Mediated Seed Dormancy	Pei et al (1998) Science 282: 287-290; Cutler et al. (1996) Science 273: 1239
Metabolic activity	Nitrogen metabolism	Goupil et al (1998) J Exptl Botany 49:1855-62
Metabolic activity	-H <sup>+</sup> export and membrane hyperpolarization	Cerana et al. (1983)
Metabolic activity	Chloroplast functioning	Benkova et al (1999) Plant

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS ALTERED	CITATIONS INCLUDING ASSAYS
		Physiol 121: 245-252
Growth, Differentiation and development	Regulation of Morphogenesis	Riou-Khamlichi et al. (1999) Science 283: 1541-44
Metabolic activity	Cell Death	Lohman et al. (1994) Physiol Plant 92: 322-328
Growth and development	Promotion of cell division Shoot formation in absence of exogenous cytokinin	Kakimoto (1996) Science 274: 982-985
Metabolic activity	Membrane repair	Heath et al. (1986) Planta 169: 304-12 Browse et al. (1986) Anal Biochem 152: 141-5 D'Aoust et al (1999) Plant Cell 11: 2407-18
Metabolism	Organic molecule export	Moody et al. (1988) Phytochemistry 27: 2857-61
Metabolic activity	Nutrient Uptake	Uozumio et al. (2000) Plant Physiol 122: 1249-59
Metabolic activity	Ion export	Uozumi et al. (2000) Plant Physiol 122: 1249-59 Frachisse et al. (2000) Plant J 21: 361-71
Growth, Differentiation and development	Division and/or elongation	Zhang and Forde (1998) Science 279: 407-409. Coruzzi et al. U.S. Pat. No. 5,955,651



PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS ALTERED	CITATIONS INCLUDING ASSAYS
Metabolic activity	Regulation of Molecular chaperones	Wisniewski et al. (1999) Physiolgia Plantarum 105: 600-608
Metabolic activity	Reactivation of Aggregation and Protein Folding	Lee and Vierling (2000) Plant Physiol. 122: 189-197
Metabolic activity	Maintenance of Native Conformation (cytosolic proteins)	Queitsch et al. (2000) The Plant Cell 12: 479-92
Metabolic activity	Regulation of Translational Efficiency	Wells et al. (1998) Genes and Development 12: 3236-51
Metabolic activity	DNA Repair	Bewley (1997) Plant Cell 9: 1055-66
Metabolic activity	Protein Synthesis using stored or newly synthesized mRNAs	Heath et al. (1986) Planta 169: 304-12
Metabolic activity	Mitochondrial repair and synthesis	MacKenzie and McIntosh (1999) Plant Cell 11: 571-86
Metabolic activity	Commencement of respiration	Debeaujon et al. (2000) Plant Physiol 122: 403-4132
	Water Uptake	Debeaujon et al. (2000) Plant Physiol 122: 403-4132

Other biological activities that are modulated by the imbibition-activated polynucleotides and polynucleotide products are listed in the Reference Tables. Assays for detecting such biological activities are described in the Table below as well as in the Domain section of the Reference Table.

D.2.d. USE OF IMBIBITION AND GERMINATION GENES TO MODULATE THE  
TRANSCRIPTION LEVELS OF OTHER GENES

The expression of many genes is "upregulated" or "downregulated" during imbibition and germination because some imbibition and germination genes are integrated into complex networks that regulate transcription of many other genes. Some imbibition and germination genes are therefore useful for regulating other genes and hence complex phenotypes.

Imbibition-activated polynucleotides may also be differentially transcribed in response to fluctuating developmental-specific polynucleotide levels or concentrations, whether internal or external to a cell, at different times during the plant life cycle to promote associated biological activities. These activities are, by necessity, a small subset of the genes involved in the development process. Furthermore, because development is a continuous process with few clear demarcations between stages, the associated metabolic and biochemical pathways overlap. Some of the changes in gene transcription are summarized in the Table below:

DEVELOPMENTAL PROCESS REGULATED BY IMBIBITION- GERMINATION GENES	PHYSIOLOGICAL/METABO LIC CONSEQUENCES OF MODIFYING GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL REGULATORY ACTIVITIES ASSOCIATED WITH IMBIBITION AND GERMINATION
Tissue Specialization  - Cotyledon Expansion - Endosperm (???) - Activation of the Shoot Apical Meristem - Activation of the Root Apical Meristem	- Lipid Catabolism - Lipoyxygenase Localization - Starch Catabolism - Seed Protein Catabolism - Growth Regulator Production, Transport, Perception, Signaling, Response (e.g., Gibberellins, Ethylene,	- Transcription Factors - Transporters - Kinases - Changes in cytoskeletal protein activity modulating cell structure - Stability of factors for protein translation - Changes in cell

<ul style="list-style-type: none"> <li>- Radicle Growth</li> <li>- Vascular System Development</li> </ul>	<p>Auxin)</p> <ul style="list-style-type: none"> <li>- Global Gene Activation</li> <li>- Transcription Initiation</li> <li>- Sucrose Synthesis and Partitioning</li> <li>- Sucrose catabolism</li> <li>- Sucrose Signaling</li> </ul>	<ul style="list-style-type: none"> <li>wall/membrane structure</li> <li>- Chromatin structure and/or DNA topology</li> <li>- Biosynthetic enzymes</li> <li>- Metabolic enzymes</li> </ul>
<p>Organelle Differentiation and Development</p>	<ul style="list-style-type: none"> <li>- Cell Wall Biosynthesis</li> <li>- Activators of Metabolic Pathways</li> <li>- Protein Remodeling</li> <li>- Cell Wall Biosynthesis</li> <li>- Membrane Repair and Synthesis</li> <li>- Specific Gene Transcription Initiation</li> <li>- Sucrose Mobilization and Partitioning</li> <li>- Sucrose Signaling</li> <li>- Activators of Metabolic Pathways</li> <li>- Auxin Perception, Response and Signaling</li> <li>- Protein Remodeling</li> <li>- Lipid Mobilization, Metabolism and Biosynthesis</li> <li>- Protein Transport, Metabolism, and Biosynthesis</li> </ul>	<ul style="list-style-type: none"> <li>- Transcription Factors</li> <li>- Transporters</li> <li>- Kinases</li> <li>- Chaperones</li> <li>- Changes in cytoskeletal protein activity modulating cell structure</li> <li>- Stability of factors for protein translation</li> <li>- Changes in cell wall/membrane structure</li> <li>- Chromatin structure and/or DNA topology</li> <li>- Biosynthetic enzymes</li> <li>- Metabolic enzymes</li> </ul>

DNA Repair	<ul style="list-style-type: none"> <li>- Cell Division</li> <li>- Cell Cycle Control</li> <li>- DNA Replication</li> <li>- Specific Gene Transcription Initiation</li> <li>- Protein Remodeling</li> <li>- Protein Synthesis</li> <li>- Repressors of Senescence</li> </ul>	<ul style="list-style-type: none"> <li>- Transcription Factors</li> <li>- Transporters</li> <li>- Kinases</li> <li>- Chaperones for protein translation</li> <li>- Changes in cell wall/membrane structure</li> <li>- Chromatin structure and/or DNA topology</li> <li>- Biosynthetic enzymes</li> </ul>
Cellular Metabolism	<ul style="list-style-type: none"> <li>- Lipid Catabolism                             <ul style="list-style-type: none"> <li>- oxidation</li> <li>- Glyoxylate cycle</li> <li>- Citric acid cycle</li> <li>- Gluconeogenesis</li> <li>- Sucrose Synthesis and Partitioning</li> </ul> </li> <li>- Starch Catabolism</li> <li>- Seed Protein Catabolism                             <ul style="list-style-type: none"> <li>- Asparagine Synthesis and Transport</li> </ul> </li> <li>- Sucrose catabolism</li> <li>- Sucrose Signaling</li> <li>- Ribosome/polysome production and maintenance</li> <li>- Housekeeping genes</li> <li>- Respiration</li> <li>- Photosynthesis</li> </ul>	<ul style="list-style-type: none"> <li>- Transcription Factors</li> <li>- Transporters</li> <li>- Kinases</li> <li>- Chaperones</li> <li>- Translation Initiation Factors</li> <li>- Biosynthetic Enzymes</li> <li>- Metabolic Enzymes</li> </ul>

Changes in the processes of germination are the result of modulation of the activities of one

or more of these many germination genes and gene products. These genes and/or products are responsible for effects on traits such as fast germination, plant vigor and seed yield, especially when plants are growing in the presence of biotic or abiotic stresses or when they are growing in barren conditions or soils depleted of certain minerals.

5           Germination genes and gene products can act alone or in combination as described in the introduction. Of particular interest are combination of these genes and gene products with those that modulate stress tolerance and/or metabolism. Stress tolerance and metabolism genes and gene products are described in more detail in the sections below.

10           Use of Promoters of Imbibition and Germination Genes

These promoters can be used to control expression of any polynucleotide, plant or non-plant, in a plant host. Selected promoters when operably linked to a coding sequence can direct synthesis of the protein in specific cell types or to loss of a protein product, for example when the coding sequence is in the antisense configuration. They are thus useful in controlling changes in imbibition and germination phenotypes or enabling novel proteins to be made in germinating seeds.

I.D.2. EARLY SEEDLING-PHASE SPECIFIC RESPONSIVE GENES, GENE  
COMPONENTS AND PRODUCTS

One of the more active stages of the plant life cycle is a few days after germination is  
complete, also referred to as the early seedling phase. During this period the plant begins  
development and growth of the first leaves, roots, and other organs not found in the embryo.  
Generally this stage begins when germination ends. The first sign that germination has been  
completed is usually that there is an increase in length and fresh weight of the radicle.

a) IDENTIFICATION OF EARLY SEEDLING PHASE GENES,  
GENE COMPONENTS AND PRODUCTS

These genes defined and identified herein are capable of modulating one or more  
processes of development and growth of many plant organs as described below. These genes  
and gene products can regulate a number of plant traits to modulate yield. Examples of such  
early seedling phase genes and gene products are shown in the Reference and Sequence, Knock-  
in, Knock-out and MA-diff Tables. The functions of the protein of some of these genes are also  
given in these Tables.

Early Seedling Genes Identified by Phenotypic Observations

Some early seedling genes were discovered and characterized from a much larger set of  
genes by experiments designed to find genes that cause phenotypic changes in germinating seeds  
as the transitioned into seedlings.

In these experiments, leaf genes were identified by either (1) ectopic expression of a  
cDNA in a plant or (2) mutagenesis of the plant genome. The plants were then cultivated and  
one or more of the following leaf phenotypes, which varied from the parental "wild-type", were  
observed:

- Abnormal growth
- Abnormal cotyledons or root growth
  - Reduced growth
  - Abnormal first leaf
  - Abnormal hypocotyl

- Abnormal pigmentation

The genes identified by these phenotypes are given in the Knock-in and Knock-out Tables.

5           Early Seedling Phase Genes Identified By Differential Expression

Such genes are active or potentially active to a greater extent in developing and rapidly growing cells, tissues and organs, as exemplified by development and growth of a seedling 3 or 4 days after planting a seed. These genes herein were also discovered and characterized from a much larger set of genes in experiments designed to find genes. Early seedling phase genes were identified by measuring the relative levels of mRNA products in a seedling 3 or 4 days after planting a seed versus a sterilized seed. Specifically, mRNA was isolated from aerial portion of a seedling 3 or 4 days after planting a seed and compared to mRNA isolated from a sterilized seed utilizing microarray procedures. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: Sqn (relating to SMD 7133, SMD 7137)). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Early Seedling Phase genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

Early Seedling Phase Genes Identified By Cluster Analyses Of Differential Expression

Early Seedling Phase Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Early Seedling Phase genes is any group in the MA\_clust

that comprises a cDNA ID that also appears in Expt ID Sqn (relating to SMD 7133, SMD 7137) of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

5                   Early Seedling Phase Genes Identified By Correlation To Genes That Cause  
                    Physiological Consequences

10                   Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Early Seedling Phase genes. A group in the MA\_clust is considered a Early Seedling Phase pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

15                   Early Seedling Phase Genes Identified By Amino Acid Sequence Similarity

20                   Early Seedling Phase genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Early Seedling Phase genes. Groups of Early Seedling Phase genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Early Seedling Phase pathway or network is a group of proteins that also exhibits Early Seedling Phase functions/utilities.

25                   Of particular interest are early seedling phase genes that are differentially expressed 3 or 4 days after planting a seed but not differentially expressed germinating seeds and/or mature leaves.

                    Examples of phenotypes, biochemical activities, and transcription profiles that can be modulated by these genes and gene products are described above and below.

30                   D.5.b. USE OF EARLY SEEDLING GENES, GENE COMPONENTS AND  
                    PRODUCTS TO MODULATE PHENOTYPES

                    Rapid, efficient establishment of a seedling is very important in commercial agriculture and horticulture. It is also vital that resources are approximately partitioned between shoot and root to facilitate adaptive growth. Phototropism and geotropism need to be established. All these require post-germination process to be sustained to ensure that vigorous seedlings are produced. Early seedling phase genes, gene components and products are useful to manipulate these and other processes.



I. Development

The early seedling phase genes, gene components and products of the instant invention are useful to modulate one or more processes of the stages of leaf morphogenesis including: stage 1- organogenesis that gives rise to the leaf primordium; stage 2- delimiting basic morphological domains; and stage 3- a coordinated processes of cell division, expansion, and differentiation. Early seedling phase genes include those genes that terminate as well as initiate leaf development. Modulating any or all of the processes leads to beneficial effects at specific locations .

A. Gene Sequences Affecting Types of Leaves

Applicants provide with these genes, gene components and gene products the means to modulate one or more of the following types of leaves, and stem:

5. Cotyledons
6. Major Leaves

B. Gene Sequences Affecting Cell properties

These genes, gene components and gene products are useful to modulate changes in:

10. Cell size
11. Cell division, rate and direction
12. Cell elongation
13. Cell differentiation
14. Xylem and phloem cell numbers
15. Cell wall composition
16. All cell types

C. Gene Sequences Affecting Leaf Architecture:

The following properties of a leaf are useful to modulate to change overall leaf architecture:

4. Veination – Improvements in photosynthetic efficiency, stress tolerance efficiency of solute and nutrient movement to and from the leaf are accomplished by increases or decreases in:
  - (c) Vein placement

- (d) Number of cells in the vein
- 5. Shape
  - (c) Elongated versus rounded
  - (d) Symmetry, around either
    - abaxial-adaxial (dorsiventral) axis
    - apical-basal (proximodistal) axis
    - margin-blade-midrib (lateral) axis

#### H. Genes Sequences Influencing Leaf Responses

Shoot apical meristem cells differentiate to become leaf primordia that eventually develop into leaves. The genes, gene components and gene products of this invention are useful to modulate any one or all of these growth and development processes, by affecting timing and rate or planes of cell divisions for example, in response to the internal plant stimuli and/or programs listed below:

- 8. Embryogenesis
- 9. Germination
- 10. Hormones
  - (b) Auxin (for more details see the section on auxin-responsive genes)
- 11. Phototropism
- 12. Coordination of leaf growth and development with that of other organs
  - (f) Roots
  - (g) Stems
- 13. Stress-related programs

#### II. Interaction with the Environment

Successful seedling establishment demands successful interaction with the environment in the soil. Early vegetation genes orchestrate and respond to interactions with the environment. Thus early seedling phase genes are useful for improving interactions between a plant and the environment that include:

- N. Pigment accumulation (see the section on Viability genes for more detail)
- O. Oxygen gain/loss control
- P. Carbon dioxide gain/loss control

- Q. Water gain/loss control
- R. Nutrient transport
- S. Light harvesting
- T. Chloroplast biogenesis
- U. Circadian rhythm control
- V. Light/dark adaptation
- W. Defense systems against biotic and abiotic stresses
- X. Metabolite accumulation
- Y. Secondary metabolite production

### III. Organizing Tissues for Photosynthesis and Metabolism

Following germination and utilization of seed reserves, plant tissues prepare for photosynthesis and seedling metabolism. Leaf meristems, and root meristems participate in these changes before cell differentiation. Many of the uses for plants depend on the success of leaves as the powerhouses for plant growth, their ability to withstand stresses and their chemical composition. Leaves are organs with many different cell types and structures. Most genes of a plant are active in leaves and therefore leaves have very diverse of pathways and physiological processes. Examples of such pathways and processes that are modulated by early seedling phase genes, gene components and products include:

- X. Photosynthesis
- Y. Sugar metabolism
- Z. Starch synthesis
- AA. Starch degradation
- BB. Nitrate and ammonia metabolism
- CC. Amino acid biosynthesis, transport
- DD. Protein biosynthesis
- EE. DNA replication, repair
- FF. Lipid biosynthesis and breakdown
- GG. Protein biosynthesis, storage and breakdown
- HH. Nucleotide transport and metabolism
- II. Cell envelope biogenesis

- JJ. Membrane formation
- KK. Mitochondrial and chloroplast biogenesis
- LL. Transcription and RNA metabolism
- MM. Vitamin biosynthesis
- 5 NN. Steroid and terpenoid biosynthesis
- OO. Devise secondary metabolite synthesis
- PP. Co-enzyme metabolism
- QQ. Flavonoid biosynthesis and degradation
- RR. Synthesis of waxes
- SS. Glyoxylate metabolism
- 10 TT. Hormone perception and response pathways

Uses of Plants that Are Modified as Described above

Altering leaf genes or gene products in a plant modifies one or more the following plant traits, to make the plants more useful for specific purposes in agriculture, horticulture and for the production of valuable molecules. The useful plants have at least one of the following:

- A. More seedling vigor
- B. A higher yield of early leaves and their molecular constituents due to different:
  - 6. Number, size, weight, harvest index
  - 7. Composition including and amounts and types of carbohydrates, proteins, oils, waxes, etc.
  - 8. Photosynthetic efficiency e.g. reduced photorespiration
  - 9. Absorption of water and nutrients to enhance yields, including
  - 25 under stresses such as high light, herbicides, and heat.
  - 10. Pathways to accumulate new valuable molecules.
- I. More optimal leaf shape and architecture in early seedling— enhancing photosynthesis and enhancing appeal in ornamental species
  - (e) size
  - 30 (f) number
  - (g) pigment

- J. A better overall plant architecture – enhancing photosynthesis and enhancing appeal in ornamental species
- K. Reduced negative effects of high planting density, by altering leaf placement to be more vertical instead of parallel to the ground, for instance

- L. Better stress tolerance, including without limitation
  - 3. Drought resistance, by decreasing water loss, for example
  - 4. Pathogen resistance

- M. Better overall yield and vigor

Plant yield of biomass and of constituent molecules and plant vigor are modulated to create benefits by genetically changing:

- 3. Growth rate of
  - (h) Seedling
  - (i) Coleoptile elongation
  - (j) Young leaves

To change any of the phenotype(s) above, activities of one or more of the early seedling phase genes or gene products are modulated in an organism and the consequence evaluated by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels are altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (Methods. Mol. Biol. 82:259-266 (1998)) with leaf gene constructs and/or screened for variants as in Winkler et al., Plant Physiol. 118: 743-50 (1998) and visually inspected for the desired phenotype and metabolically and/or functionally assayed for altered levels of relevant molecules.

#### D.5.c. USE OF EARLY SEEDLING PHASE GENES, GENE COMPONENTS AND PRODUCTS TO MODULATE BIOCHEMICAL ACTIVITIES

Seedlings are complex and their structure, function and properties result from the integration of many processes and biochemical activities. Some of these are known from the published literature and some can be deduced from the genes and their products described in this application. Early seedling phase genes, and gene components are used singly or in combination

to modify these processes and biochemical activities and hence modify the phenotypic and trait characteristics described above. Examples of the processes and metabolic activities are given in the Table below. The resulting changes are measured according to the citations included in the Table.

5

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Metabolism -- anabolic and catabolic	G. Farnesyl ation	Pei et al., <u>Science</u> <u>282</u> : 287-290 (1998); Cutler et al., <u>Science</u> <u>273</u> : 1239 (1996)
	H. Cell Wall Biosynt hesis	Goupil et al., <u>J Exptl. Botany</u> <u>49</u> :1855-62 (1998) Walch-Liu et al., <u>J Exppt. Botany</u> <u>51</u> , 227-237 (2000)
	I. Nitrogen Metabol ism	
	J. Seconda ry Metabol ite Biosynt hesis and Degrada tion	
Water Conservation And Resistance To Drought	A. Production of polyols	Allen et al., <u>Plant Cell</u> <u>11</u> : 1785- 1798 (1999)

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
And Other Related Stresses	B. Regulation of salt concentration C. ABA response(s)	Li et al., <u>Science</u> <u>287</u> : 300-303 (2000) Burnett et al., <u>J Exptl. Botany</u> <u>51</u> : 197-205 (2000) Raschke, In: <u>Stomatal Function</u> , Zeiger et al. Eds., 253-279 (1987)
Transport Anion and Cation Fluxes	(i) Ca <sup>2+</sup> Accumu lation (a) K <sup>+</sup> Fluxes (b) Na <sup>+</sup> Fluxes 1. Receptor – ligand binding 2. Anion and Cation fluxes	Lacombe et al., <u>Plant Cell</u> <u>12</u> : 837- 51 (2000); Wang et al., <u>Plant Physiol.</u> <u>118</u> :1421-1429 (1998); Shi et al., <u>Plant Cell</u> <u>11</u> : 2393- 2406 (1999) Gaymard et al., <u>Cell</u> <u>94</u> :647-655 (1998) Jonak et al., <u>Proc. Natl. Acad. Sci.</u> <u>93</u> : 11274-79 (1996); Sheen, <u>Proc. Natl. Acad. Sci.</u> <u>95</u> : 975-80 (1998); Allen et al., <u>Plant Cell</u> <u>11</u> : 1785-98 (1999)
Carbon Fixation	3. Calvin Cycle 5. Photorespiratio n 6. Oxygen	Wingler et al., <u>Philo Trans R Soe</u> <u>Lond B Biol Sci</u> <u>355</u> , 1517-1529 (2000);

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	<p>evolution</p> <p>7. RuBisCO</p> <p>4. Chlorophyll metabolism</p> <p>(ii) Chloropl ast Biogene sis and Metabol ism</p> <p>5. Fatty Acid and Lipid Biosynthesis</p> <p>(iii) Glyoxyl ate metaboli sm</p> <p>(iv) Sugar Transpo rt</p> <p>(v) Starch Biosynt hesis and Degrada</p>	<p>Palecanda et al., <u>Plant Mol Biol</u> <u>46</u>, 89-97 (2001);</p> <p>Baker et al., <u>J Exp Bot</u> <u>52</u>, 615-621 (2001)</p> <p>Chen et al., <u>Acta Biochim Pol</u> <u>41</u>, 447-457 (1999)</p> <p>Imlau et al., <u>PlantCell II</u>, 309-322 (1999)</p>

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PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	tion	
Hormone Perception and Growth	<p>(vi) Hormone Receptors and Downstream Pathways for</p> <p>(a) ethylene</p> <p>(b) jasmonic acid</p> <p>(c) brassinosteroid</p> <p>(d) gibberellin</p> <p>(e) auxin</p> <p>(f) cytokinin</p> <p>- Activation Of Specific Kinases And Phosphatases</p>	<p>Tieman et al., <u>Plant J</u> <u>26</u>, 47-58 (2001)</p> <p>Hilpert et al., <u>Plant J</u> <u>26</u>, 435-446 (2001)</p> <p>Wenzel et al., <u>Plant Phys</u> <u>124</u>, 813-822 (2000)</p> <p>Dengler and Kang, <u>Curr Opin Plant Biol</u> <u>4</u>, 50-56 (2001)</p> <p>Tantikanjana et al., <u>Genes Dev</u> <u>15</u>, 1577-1580 (2001)</p>
See Imbibition, Shoot Apical Meristem, Root and Leaf sections for more details		

Other biological activities that are modulated by the leaf genes and gene products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table, for example.

D.5.d. USE OF EARLY SEEDLING PHASE GENES, GENE COMPONENTS  
AND PRODUCTS TO MODULATE TRANSCRIPTION LEVELS

The expression of many genes is "up regulated" or down regulated" in plants because  
5 some genes and their products are integrated into complex networks that regulate transcription of  
many other genes. Some early seedling phase genes, gene components and products are  
therefore useful for modifying the transcription of other genes and hence complex phenotypes, as  
described above. Profiles of leaf gene activities are described in the Table below with associated  
biological activities. "Up-regulated" profiles are those where the mRNA transcript levels are  
10 higher in young seedlings as compared to the sterilized seeds. "Down-regulated" profiles  
represent higher transcript levels in the plantlet as compared to sterilized seed only.

I.D.3. SIZE AND STATURE GENES, GENE COMPONENTS AND  
PRODUCTS

Great agronomic value can result from modulating the size of a plant as a whole or of any of  
its organs. For example, the green revolution came about as a result of creating dwarf wheat plants,  
which produced a higher seed yield than taller plants because they could withstand higher levels and  
inputs of fertilizer and water. Size and stature genes elucidated here are capable of modifying the  
growth of either an organism as a whole or of localized organs or cells. Manipulation of such  
15 genes, gene components and products can enhance many traits of economic interest from increased  
seed and fruit size to increased lodging resistance. Many kinds of genes control the height attained  
by a plant and the size of the organs. For genes additional to the ones in this section other sections  
of the Application should be consulted.

a) Identification of Size and Stature Genes, Gene Components and  
Products

Size and stature genes identified herein are defined as genes, gene components and  
products capable of modulating one or more processes in growth and development, to produce  
changes in size of one or more organs. Examples of such stature genes and gene products are  
20 shown in the Reference, Sequence, Protein Group, Protein Group Matrix, Knock-in, Knock-out,  
30

MA-diff and MA-clust. The biochemical functions of the protein products of many of these genes determined from comparisons with known proteins are also given in the Reference tables.

b) Size And Stature Genes, Gene Components And Products  
Identified By Phenotypic Observations

Mutant plants exhibiting increased or decreased stature in comparison to parental wild-type plants were used to identify size and stature genes. In these experiments, size and stature genes were identified by either (1) the ectopic expression of a cDNA in a plant or (2) mutagenesis of the plant genome. The plants were then cultivated and stature genes were identified from plants that were smaller than the parental "wild-type". The phenotypes and gene mutations associated with them are given in Tables

Examples of phenotypes, biochemical activities, or transcript profiles that are modulated using these genes are described above and below.

c) Use Of Size And Stature Genes, Gene Components And Products  
To Modulate Phenotypes

Typically, these genes can cause or regulate cell division, rate and time; and also cell size and shape. Many produce their effects via meristems. These genes can be divided into three classes. One class of genes acts during cytokinesis and/or karyokinesis, such as mitosis and/or meiosis. A second class is involved in cell growth; examples include genes regulating metabolism and nutrient uptake pathways. Another class includes genes that control pathways that regulate or constrain cell division and growth. Examples of these pathways include those specifying hormone biosynthesis, hormone sensing and pathways activated by hormones.

Size and stature genes and gene components are useful to selectively alter the size of organs and stems and so make plants specifically improved for agriculture, horticulture and other industries. There are a huge number of utilities. For example, reductions in height of specific ornamentals, crops and tree species can be beneficial, while increasing height of others may be beneficial.

Increasing the length of the floral stems of cut flowers in some species would be useful, while increasing leaf size in others would be economically attractive. Enhancing the size of

specific plant parts, such as seeds, to enhance yields by stimulating hormone (Brassinolide) synthesis specifically in these cells would be beneficial. Another application would be to stimulate early flowering by altering levels of gibberellic acid in specific cells. Changes in organ size and biomass also results in changes in the mass of constituent molecules. This makes the utilities of size and stature genes useful for the production of valuable molecules in parts of plants, for extraction by the chemical and pharmaceutical industries.

Examples of phenotypes that can be modulated by the genes and gene components are described above and below:

I. Cellular Level:

Size and stature genes and gene products can be used to modulate cellular changes in:

- A. Cell size
- B. Cell shape
- C. Cell division, rate and direction
- D. Cell elongation
- E. Cell differentiation
- F. Stomata number
- G. Trichome number

II. Organ Level

The genes of the invention are useful to regulate the development and growth of:

- A. Roots
  - 1. Primary
  - 2. Lateral
  - 3. Root hairs
  - 4. Root cap
  - 5. Apical meristem
  - 6. Epidermis
  - 7. Cortex
  - 8. Stele
- B. Stem

1. Pholem
2. Xylem
3. Nodes
4. Internodes
5. Shoot apical meristem

C. Leaves

1. Cauline
2. Rosette
3. Petioles

D. Flowers

1. Receptacle
2. Sepals, Petals, and Tepals
  - (a) Color
  - (b) Shape
  - (c) Size
  - (d) Number
  - (e) Petal Drop
3. Androecium
4. Stamen
5. Anther
6. Pollen
7. Sterility
8. Size, shape, weight, color
9. Filament
10. Gynoecium
11. Carpel
12. Ovary
13. Style
14. Stigma
15. Ovule
  - (a) Size, shape, number

16. Pedicel and Peduncle
17. Flowering time
18. Fertilization

E. Seeds

1. Placenta
2. Embryo
3. Cotyledon
4. Endosperm
5. Suspensor
6. Seed coat (testa)

F. Fruits

1. Pericarp – thickness, texture
2. Exocarp
3. Mesocarp
4. Endocarp

III. Overall Organism Level

The following traits can be modulated with the genes and gene products of this invention to affect the traits of a plant as a whole:

A. Architecture

1. Branching
2. Ornamental architecture
3. Shade avoidance
4. Planting density effects
5. Wind resistance

B. Vigor

1. Increased biomass
2. Drought tolerance

To regulate any of the phenotype(s) above, activities of one or more of the sizing genes or gene products are modulated in an organism and tested by screening for the desired trait.

Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (Methods. Mol. Biol. 82:259-266 (1998)) and/or screened for variants as in Winkler et al., (Plant Physiol. 118: 743-50 1998) and visually inspected for the desired phenotype or metabolically and/or functionally assayed.

D.3.b. USE OF SIZE AND STATURE GENES, GENE COMPONENTS AND PRODUCTS TO MODULATE BIOCHEMICAL ACTIVITIES

Many metabolic and developmental processes can be modulated by size and stature genes and gene components to achieve the phenotypic characteristics exemplified above. Some of these are listed below. Such biological activities can be measured according to the citations included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Growth and Development	Gibberellic Acid Biosynthesis Gibberellic Acid Receptor and Downstream Pathways	Swain SM, Tseng Ts, Olszewski NE. Altered expression of spindly affects gibberellin response and plant development. <i>Plant Physiol</i> 2001 Jul;126(3):1174-85  Hooley, R. Gibberellins: perception, transduction, and responses. <i>Plant Mol. Biol.</i> 1994 26:1529-1555.  Hooley, R. Gibberellins: perception, transduction, and responses. <i>Plant Mol. Biol.</i> 1994 26:1529-1555.  Perata, P, Matsukura, C, Vernieri, P, Yamaguchi, J, Sugar repression of a gibberellin-dependent signaling pathway in barley embryos. <i>Plant Cell</i> 1997

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	Brassinolide Biosynthesis Brassinolide Receptors, Degradation of Brassinolide Pathways affected by Brassinolide	9:2197-2208.  Noguchi T, Fujioka S, Choe S, Takatsuto S, Tax FE, Yoshida S, Feldmann KA. Biosynthetic pathways of brassinolide in Arabidopsis. Plant Physiol 2000 Sep;124(1):201-9  Wang ZY, Seto H, Fujioka S, Yoshida S, Chory J. BRI1 is a critical component of a plasma-membrane receptor for plant steroids. Nature 2001 Mar 15;410(6826):380-3  Neff MM, Nguyen SM, Malancharuvil EJ, Fujioka S, Noguchi T, Seto H, Tsubuki M, Honda T, Takatsuto S, Yoshida S, Chory J. BAS1: A gene regulating brassinosteroid levels and light responsiveness in Arabidopsis. Proc Natl Acad Sci U S A 1999 Dec 21;96(26):15316-23  Kang JG, Yun J, Kim DH, Chung KS, Fujioka S, Kim JI, Dae HW, Yoshida S, Takatsuto S, Song PS, Park CM. Light and brassinosteroid signals are integrated via a dark-induced small G protein in etiolated seedling growth. Cell 2001 Jun 1;105(5):625-36
	Cytokinin biosynthesis Cytokinin receptor Degradation of Cytokinin Pathways affected by Cytokinin	Mok DW, Mok MC. Cytokinin metabolism and action. Annu Rev Plant Physiol Plant Mol Biol 2001;52:89-118  Schmulling T. CREAm of



PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	Auxin Biosynthesis Auxin Receptor Auxin Degradation Pathways affected by Auxins Auxin transport	<p>cytokinin signalling: receptor identified. Trends Plant Sci 2001 Jul;6(7):281-4</p> <p>Mok DW, Mok MC. Cytokinin metabolism and action. Annu Rev Plant Physiol Plant Mol Biol 2001;52:89-118</p> <p>Seyedi M, Selstam E, Timko MP, Sundqvist C. The cytokinin 2-isopentenyladenine causes partial reversion to skotomorphogenesis and induces formation of prolamellar bodies and protochlorophyllide657 in the lip1 mutant of pea. Physiol Plant 2001 Jun;112(2):261-272</p> <p>Zhao Y, Christensen SK, Fankhauser C, Cashman JR, Cohen JD, Weigel D, Chory J. A role for flavin monooxygenase-like enzymes in auxin biosynthesis. Science 2001 Jan 12;291(5502):306-9</p> <p>Abel S, Ballas N, Wong LM, Theologis A. DNA elements responsive to auxin. Bioessays 1996 Aug;18(8):647-54</p> <p>del Pozo JC, Estelle M. Function of the ubiquitin-proteasome pathway in auxin response. Trends Plant Sci 1999 Mar;4(3):107-112.</p> <p>Rahman A, Amakawa T, Goto N, Tsurumi S. Auxin is a positive regulator for ethylene-mediated response in the</p>

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
		<p>growth of Arabidopsis roots. Plant Cell Physiol 2001 Mar; 42(3):301-7</p> <p>Zhao Y, Christensen SK, Fankhauser C, Cashman JR, Cohen JD, Weigel D, Chory J. A role for flavin monooxygenase-like enzymes in auxin biosynthesis. Science 2001 Jan 12;291(5502):306-9</p> <p>Abel S, Ballas N, Wong LM, Theologis A. DNA elements responsive to auxin. Bioessays 1996 Aug;18(8):647-54</p> <p>del Pozo JC, Estelle M. Function of the ubiquitin- proteasome pathway in auxin response. Trends Plant Sci 1999 Mar;4(3):107-112.</p> <p>Rahman A, Amakawa T, Goto N, Tsurumi S. Auxin is a positive regulator for ethylene- mediated response in the growth of Arabidopsis roots. Plant Cell Physiol 2001 Mar; 42(3):301-7</p> <p>Gil P, Dewey E, Friml J, Zhao Y, Snowden KC, Putterill J, Palme K, Estelle M, Chory J. BIG: a calossin-like protein required for polar auxin transport in Arabidopsis. Genes Dev. 2001 Aug 1;15(15):1985-97</p> <p>Estelle M., Polar auxin transport. New support for an old model. Plant Cell 1998 Nov;10(11):1775-8</p>

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PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	Cell wall growth	Cosgrove DJ., Loosening of plant cell walls by expansins. Nature 2000 Sep 21;407(6802):321-6

Other biological activities that are modulated by the stature genes and gene products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table, for example.

Changes in the size, vigor, or yield of a plant are the result of modulation of the activities of one or more of these many size and stature genes and gene products. While size and stature polynucleotides and gene products can act alone, combinations of these polynucleotides and also with others that also affect growth and development are especially useful.

#### Use of Promoters of "Size and Stature" Genes

Promoters of "size and stature" genes are useful for controlling the transcription of any desired polynucleotides, both plant and non-plant. They can be discovered from the "size and stature" genes in the Reference Tables, and their patterns of activity from the MA Tables. When operably linked to any polynucleotide encoding a protein, and inserted into a plant, the protein will be synthesized in those cells in which the promoter is active. Many "size and stature" genes will function in meristems, so the promoters will be useful for expressing proteins in meristems. The promoters can be used to cause loss of, as well as synthesis of, specific proteins via antisense and sense suppression approaches.

I.D.4. SHOOT-APICAL MERISTEM GENES, GENE COMPONENTS AND  
PRODUCTS

5 New organs, stems, leaves, branches and inflorescences develop from the stem apical  
meristem (SAM). The growth structure and architecture of the plant therefore depends on the  
behavior of SAMs. Shoot apical meristems (SAMs) are comprised of a number of morphologically  
undifferentiated, dividing cells located at the tips of shoots. SAM genes elucidated here are capable  
of modifying the activity of SAMs and thereby many traits of economic interest from ornamental  
leaf shape to organ number to responses to plant density.

10 a) IDENTIFICATION OF SAM GENES, GENE COMPONENTS  
AND PRODUCTS

15 SAM genes identified herein are defined as genes, gene components and products capable of  
modulating one or more processes or functions of SAMs as described below. Regulation of SAM  
genes and gene products are useful to control many plant traits including architecture, yield and  
vigor. Examples of such SAM genes and gene products are shown in the Reference, Sequence,  
Protein Group, Protein Group Matrix, phenotype and MA-diff Tables. The functions of many of the  
protein products of these genes are also given in the Reference tables.

20 Sam Genes, Gene Components And Products Identified By Phenotypic Observations

25 SAM genes were discovered and characterized from a much larger set of genes by  
experiments designed to find genes that cause phenotypic changes in leaf morphology, such as  
cotyledon or leaf fusion. In these experiments, SAM genes were identified by either (1) ectopic  
expression of a cDNA in a plant or (2) mutagenesis of the plant genome. The plants were then  
cultivated and one or more of the following phenotypes, which varied from the parental "wild-  
type", was observed:

- I. Cotyledon
  - Fused
- II. Leaves
  - Fused

- Leaf placement on stems
- III. Branching
  - Number
- IV. Flowers
  - Petals fused
  - Altered bolting
  - Early bolting
  - Late bolting
  - Strong bolting
  - Weak bolting
  - Abnormal branching

For more experimental detail see the Example section below. The genes identified by these results of the phenotypes that are shown in Knock-in and Knock-out Tables.

#### Sam Genes, Gene Components And Products Identified By Differential Expression

SAM genes were also identified in experiments designed to find genes whose mRNA products are associated specifically or preferentially with SAMs. The concentration of mRNA products in the arabidopsis plant with the SHOOTMERISTEMLESS (STM) gene knocked-out was measured relative to the concentration in the parental, non-mutant plant. The Arabidopsis STM gene is required for embryonic SAM formation. The STM gene encodes a Knotted1 (Kn1) type of homeodomain protein. Homeodomain proteins regulate transcription of many genes in many species and have been shown to play a role in the regulation of translation as well. Seedlings homozygous for recessive loss-of-function alleles germinate with roots, a hypocotyl, and cotyledons, but no SAM is formed. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108478, 108479, 108480, 108481, 108598, 108535, 108536, 108435). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Meristem genes are those sequences that showed differential expression as compared to

controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

Meristem Genes Identified By Cluster Analyses Of Differential Expression

Meristem Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Meristem genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108478, 108479, 108480, 108481, 108598, 108535, 108536, 108435 of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

Meristem Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Meristem genes. A group in the MA\_clust is considered a Meristem pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

Meristem Genes Identified By Amino Acid Sequence Similarity

Meristem genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Meristem genes. Groups of Meristem genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Meristem pathway or network is a group of proteins that also exhibits Meristem functions/utilities.

Examples of phenotypes, biochemical activities, and transcription profiles that can be modulated by SAM genes and gene products are described above and below.

b) USE OF SAM GENES, GENE COMPONENTS AND PRODUCTS  
TO MODULATE PHENOTYPES

With the SAM genes and gene products of the invention, Applicants provide the means to modulate one or more of the following types of SAMs:

1. Embryonic meristem
2. Vegetative lateral SAMs
3. Inflorescence lateral SAMs
4. Floral meristems
5. Adventitious SAM

The SAM genes of the instant invention are useful for modulating one or more processes of SAM structure and/or function including (I) cell size and division; (II) cell differentiation and organ primordia.

I. Cell Size and Division

A. Cell Properties

SAM genes and gene products can be used to modulate changes in:

1. Cell size
2. Cell division, rate and direction
3. Cell division symmetry

A key attribute of the SAM is its capacity for self-renewal. The self-renewing initial cell population resides in the central zone of the SAM. A small number of slowly dividing initial cells (typically 2 to 4 per layer) act as a self-replenishing population, whereas some of their descendants, pushed out onto the flanks of the SAM, differentiate into leaves. Other descendants, displaced below the SAM, differentiate into stem. The immediate descendants of the initial cells divide further, amplifying the cell population before being incorporated into leaf or stem primordia.

The genes and gene components of this invention are useful for modulating any one or all of these cell division processes generally, as in timing and rate, for example. In addition, the polynucleotides and polypeptides of the invention can control the response of these processes to the internal plant programs associated with:

1. Embryogenesis

2. Hormone responses

Cytokinin (inhibitory for root development, see section on cytokinin-responsive genes)

3. Coordination of growth and development with that of other plant organs

- a. Leaves
- b. Flowers
- c. Seeds
- d. Fruits

SAM genes can also be used to control the response of these processes to changes in the environment, including heat, cold, drought, high light and nutrition.

B. Sam Cell Patterns And Organization

Although SAMs appear as small regions of morphological undifferentiated dividing cells, a group of morphologically undifferentiated dividing cells does not necessarily constitute a SAM. Rather, evidence indicates that SAMs are highly organized or patterned regions of the plant in which many important events in early organogenesis occur. Thus, the term "SAM" is used to denote a highly organized structure and site of pattern formation. The invention also permits engineering of specific as well as overall features of SAM architecture as follows:

1. Zones

- a. Central
- b. Peripheral
- c. Rib

2. Layers

- a. L1
- b. L2
- c. L3

3. Symmetry

II Cell Differentiation And Organ Primordia

The apical meristem in many species first undergoes a vegetative phase whereby cells set aside from the apex become leaf primordia with an axillary vegetative meristem. Upon floral



induction, the apical meristem is converted to an inflorescence meristem. The inflorescence meristem arises in the axils of modified leaves and is indeterminate, producing whorls or rings of floral organ primordia. In species which produce terminal flowers, the apical meristem is determinate and eventually adopts a third identity, that of a floral meristem. Examples of the plant properties that the genes and gene products of the invention can be used to modulate include:

1. Indeterminancy
  - a. Inhibiting or increasing differentiation
  - b. Enhancing plant growth and yield
2. Symmetry
  - a. Symmetry of organs developed
  - b. Symmetry of arrangement of organs, such as leaves, petals, flowers, etc.
3. Leaf fate and timing (for more detail see leaf section)
  - a. Internode length modulation
    - i. Longer internodes to increase shade avoidance
    - ii. Shorter internodes to favor leaf development
4. Floral fate and timing of flowering (for more detail see the reproduction section)

#### Uses Of Plants Modified As Described Above Using SAM Genes, Gene Components And Products

Because SAMs determine the architecture of the plant, modified plants will be useful in many agricultural, horticultural, forestry and other industrial sectors. Plants with a different shape, numbers of flowers and seed and fruits will have altered yields of plant parts. For example, plants with more branches can produce more flowers, seed or fruits. Trees without lateral branches will produce long lengths of clean timber. Plants with greater yields of specific plant parts will be useful sources of constituent chemicals. Such plants will have, for example:

- A. More prolific leaf development (see Leaf section for more detail)
- B. Better optimized stem and shoot development (see Stem section for more detail)
- C. Adventitious shoots

D. More flowers, seeds, and fruits (see Reproduction section for more detail)

E. Enhanced vigor, including growth rate of:

1. Whole plant, including height, flowering time, etc.
2. Seedling
3. Coleoptile elongation
4. Young leaves
5. Flowers
6. Seeds
7. Fruit

F. Higher yields based on:

1. Biomass

Fresh and dry weight during any time in plant life, including maturation and senescence

2. Number of flowers

3. Seed yield

- a. Number, size, weight, harvest index
- b. Content and composition, e.g. amino acid, jasmonate, oil, protein and starch

4. Fruit yield

- a. Number, size, weight, harvest index
- b. Content and composition, e.g. amino acid, jasmonate, oil, protein and starch

To regulate any of the phenotype(s) above, activities of one or more of the SAM genes or gene products can be modulated and tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266) and/or screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and visually inspected for the desired phenotype or metabolically and/or functionally assayed according to Dolan et al. (1993, Development 119: 71-84), Dolan et al. (1997, Development 124: 1789-98), Crawford and Glass

5

SAM genes and gene components are useful for modulating biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

253

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Perception	including gibberellic acid, Auxin and cytokinin.	Physiology 116(2):471-476.
	Gibberellic acid biosynthesis GA biosynthetic enzyme GA-20 oxidase is a required step in GA biosynthesis. GA-20 oxidase is Regulated by some SAM gene products.	Modulation of GA perception and function can be assayed as described in Sakamoto, T. et al. 2001 Genes and Development 15: 581-590.
	Over expression of SAM genes can lead to reduced internode elongation, reduced cell elongation and reduced cell expansion.	Sakamoto, T. et al. 2001. Genes and Development 15: 581-590.
	Cytokinin Receptor activity	Inoue, T. et al., Nature 409:1060-1063.
	SAM gene products can affect the activity of Auxin dependent postranscriptional gene protein expression.	Sieberer, T. et al., 2000 Current Biology 10:1595-1598. del Pozo, J. C.; Estelle, M. PNAS (USA) 1999. 96(26):15342-15347.
	SAM gene products can affect Auxin Perception/metabolism in the meristem to produce useful changes in plant architecture.	Tantikanjana, T. Genes and Development. June 15, 2001. 15(12):1577-1588.
Leaf senescence	SAM gene products can increase and decrease leaf senescence	Ori, N. et al; Plant Cell. June, 1999. 11(6):1073-1080.

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	rate. This can be done by modulating cytokinin hormone levels.	
	Cytokinin effect on cell division and expansion.	Beemster, Gerrit T. S.; Baskin, Tobias I. 2000 Plant Physiology 124:1718-1727.
Adventitious shoot formation	Alter growth hormone status.	Kusaba, S. et al; 1998 Plant Physiology 116(2):471-476
	Ectopic expression of SAM genes in leaf or other non SAM organs or tissue can produce shoots	Chuck, G. 1996 Plant Cell 8: 1227-1289.
	Pathways comprising isopentenyl transferase (ipt)	

Other biological activities that can be modulated by the SAM genes and gene products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table.

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d) USE OF SAM GENES, GENE COMPONENTS AND PRODUCTS  
TO MODULATE TRANSCRIPTION LEVELS OF OTHER GENES

The expression of many genes is "upregulated" or "downregulated" in the SAM mutants because some of the SAM genes are integrated into complex networks that regulate the transcription of many other genes. Some SAM genes and gene components are therefore useful for modifying the transcription of other genes and hence complex phenotypes as described above. Profiles of genes altered by SAM mutations and genes are described in the Table below with associated biological activities. "Up-regulated" profiles are for genes whose mRNA levels are higher in the

stm plants as compared to parental wild-type plants; and vice-versa for "down-regulated" profiles.

TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING SAM GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES WHOSE TRANSCRIPTS ARE CHANGED
Up Regulated Transcripts	Genes repressed by SAMs directly or indirectly	<ul style="list-style-type: none"> <li>• Altered Auxin/cytokinin hormone ratio and perception.</li> <li>• Increased/decreased cell expansion – promoting effects of brassinosteroids and gibberellic acids, due to altered levels of biosynthetic pathway enzymes and or the amount of functional hormone receptor.</li> <li>• Increased or decreased rate of cell division.</li> <li>• Altered planes of cell division</li> <li>• Increased or decreased rate and extent of cell expansion.</li> <li>• Increased or</li> </ul>	<ul style="list-style-type: none"> <li>• Transporters</li> <li>• Metabolic Enzymes</li> <li>• Cell Membrane Structure</li> <li>• Kinases, Phosphatases, G-Proteins</li> <li>• Transcription Activators/Repressors</li> <li>• Transcription coactivators/corepressor s</li> <li>• Chromatin Structure And/Or Localized DNA Topology Proteins</li> <li>• Cell Wall Proteins</li> <li>• Translational activators/repressors</li> <li>• Cell wall proteins involved in cell rigidity e.g. extensin, glycine rich proteins.</li> <li>• Cell cycle regulatory proteins such as cyclins and cyclin dependent</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING SAM GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES WHOSE TRANSCRIPTS ARE CHANGED
		decreased rigidity of cell walls.	protein kinases (CDKs).
Down-Regulated Transcripts	Genes involved in SAM cells and genes whose expression is induced by SAMs	<ul style="list-style-type: none"> <li>• Altered pattern of organs emerging from the meristem</li> <li>• Increased or decreased the number of cells partitioned into a lateral organ.</li> <li>• Altered apical dominance due to suppression of lateral bud growth.</li> <li>• Altered apical dominance due to releasing of axillary meristems from repression.</li> <li>• Increased/or decreased production of adventitious meristems.</li> <li>• Increased potential to form somatic embryos.</li> </ul>	<ul style="list-style-type: none"> <li>• Auxin transporter proteins</li> <li>• Auxin receptor proteins</li> <li>• Cytokinin receptor proteins</li> <li>• Gibberellic acid receptor proteins</li> <li>• Brassinolide receptor proteins</li> <li>• Hormone biosynthesis proteins</li> <li>• Hormone degradation proteins</li> <li>• Hormone conjugation proteins</li> <li>• Ubiquitin conjugating enzymes.</li> <li>• Receptor kinase signal transduction</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING SAM GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES WHOSE TRANSCRIPTS ARE CHANGED
		<ul style="list-style-type: none"> <li>• Altered cell signaling pathways</li> <li>• Altered hormone levels</li> </ul>	

SAM genes and gene products can be modulated alone or in combination as described in the introduction. Of particular interest are combination of these genes and gene products with those that modulate hormone responsive pathways. Hormone responsive genes and gene products are described in more detail in the sections below.

#### Use Of Sam Gene Promoters To Modify Sams

Promoters of SAM genes, as described in the Reference tables, for example, can be used to modulate transcription of coding sequences in SAM cells to influence growth, differentiation or patterning of development or any of the phenotypes or biological activities above. For example, any desired sequence can be transcribed in similar temporal, tissue, or environmentally specific patterns as a SAM gene when the desired sequence is operably linked to the promoter of the SAM gene.

A specific instance is linking of a SAM gene promoter normally active in floral meristem primordia, to a phytotoxic protein coding sequence to inhibit apical meristem switching into an inflorescence and/or floral meristem, thereby preventing flowering.

SAM gene promoters can also be used to induce transcription of antisense RNA copies of a gene or an RNA variant to achieve reduced synthesis of a specific protein in specific SAM cells. This provides an alternative way to the example above, to prevent flowering.



TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENE PRODUCTS WITH MODIFIED LEVELS
Up regulated Transcripts	<p>Genes involved in leaf, stem and root cell differentiation, cell division, cell expansion</p> <p>Genes involved in positive regulation of root, stem and leaf genes</p> <p>Repressors of root and other organ cell types e.g. flowers</p>	<ul style="list-style-type: none"> <li>• Leaf cells proliferate and differentiate;</li> <li>• Leaf structures form and expand</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription factors, signal transduction proteins, kinase and phosphatases</li> <li>• Chromatin remodeling</li> <li>• Hormone biosynthesis enzymes</li> <li>• Receptors</li> </ul>
	Genes involved in photosynthesis	<ul style="list-style-type: none"> <li>• Photosynthesis and plastid differentiation</li> <li>• Calvin cycle activated</li> <li>• Chloroplast biogenesis and plastid differentiation activated</li> </ul>	<ul style="list-style-type: none"> <li>• Light harvesting coupled to ATP production</li> <li>• Chlorophyll biosynthesis</li> <li>• Ribulose Bisphosphate carboxylase</li> <li>• Chloroplast membranes synthesis</li> <li>• Chloroplast ribosome biogenesis</li> </ul>
	Other genes involved in metabolism	<ul style="list-style-type: none"> <li>• Starch biosynthesis</li> <li>• Lipid biosynthesis</li> <li>• Nitrogen metabolism – NO<sub>3</sub> reduced and amino acids made</li> <li>• Secondary metabolites produced</li> </ul>	<ul style="list-style-type: none"> <li>• Starch synthase</li> <li>• Nitrate reductase</li> <li>• Terpenoid biosynthesis</li> <li>• Transcription factors</li> <li>• Transporters</li> <li>• Kinases</li> <li>• Phosphatases and signal transduction protein</li> <li>• Chromatin</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENE PRODUCTS WITH MODIFIED LEVELS
			structure modulators
Down regulated genes	<p>Genes involved in negative regulation of root, stem and leaf genes</p> <p>Genes involved in other organs e.g. flowers</p>	<ul style="list-style-type: none"> <li>• Leaf genes activated and leaf functions induced</li> <li>• Other organs not induced</li> <li>• Leaf, stem and root metabolic pathways induced</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription factors</li> <li>• Signal transduction proteins – kinases and phosphatases</li> <li>• Metabolic enzymes</li> <li>• Chromatin remodeling proteins</li> </ul>

While early seedling phase polynucleotides and gene products are used singly, combinations of these polynucleotides are often better to optimize new growth and development patterns. Useful combinations include different leaf polynucleotides and/or gene products with a hormone responsive polynucleotide. These combinations are useful because of the interactions that exist between hormone-regulated pathways, nutritional pathways and development.

#### Use of Early Seedling Phase Gene Promoters

Promoters of early seedling phase genes are useful for transcription of desired polynucleotides, both plant and non-plant. If the gene is expressed only in the post-germination seedling, or in certain kinds of leaf cells, the promoter is used to drive the synthesis of proteins specifically in those cells. For example, extra copies of carbohydrate transporter cDNAs operably linked to a early seedling phase gene promoter and inserted into a plant increase the “sink” strength of leaves. Similarly, early seedling phase promoters are used to drive transcription of metabolic enzymes that alter the oil, starch, protein, or fiber contents of the seedling. Alternatively, the promoters direct expression of non-plant genes that can, for instance, confer resistance to specific pathogen. Additionally the promoters are used to synthesize an antisense mRNA copy of a gene to inactivate the normal gene expression into protein. The

promoters are used to drive synthesis of sense RNAs to inactivate protein production via RNA interference.

I.D.5. VEGETATIVE-PHASE SPECIFIC RESPONSIVE GENES, GENE  
COMPONENTS AND PRODUCTS

Often growth and yield are limited by the ability of a plant to tolerate stress conditions, including water loss. To combat such conditions, plant cells deploy a battery of responses that are controlled by a phase shift, from so called juvenile to adult. These changes at distinct times involve, for example, cotyledons and leaves, guard cells in stomata, and biochemical activities involved with sugar and nitrogen metabolism. These responses depend on the functioning of an internal clock, that becomes entrained to plant development, and a series of downstream signaling events leading to transcription-independent and transcription-dependent stress responses. These responses involve changes in gene expression.

Manipulation of the activation of one or more genes controlling the phase changes are useful to modulate the biological processes and/or phenotypes listed below. Phase responsive genes and gene products can act alone or in combination. Useful combinations include phase responsive genes and/or gene products with similar transcription profiles, similar biological activities, or members of the same or functionally related biochemical pathways. Whole pathways or segments of pathways are controlled by transcription factor proteins and proteins controlling the activity of signal transduction pathways. Therefore, manipulation of such protein levels is especially useful for altering phenotypes and biochemical activities of plants.

Phase responsive genes and gene products can function to either increase or dampen the above phenotypes or activities. Characterization of phase responsive genes was carried out using microarray technology. Microarray technology allows monitoring of gene expression levels for thousands of genes in a single experiment. This is achieved by hybridizing labeled fluorescent cDNA pools to glass slides that contain spots of DNA (Schena et al. (1995) Science 270: 467-70). The US Arabidopsis Functional Genomics Consortium (AFGC) has recently made public the results from such microarray experiments conducted with AFGC chips containing about 10,000 non-redundant ESTs, selected from about 37,000 randomly sequenced ESTs generated from mRNA of different tissues and developmental stages.

The sequences of the ESTs showing at least two-fold increases or decreases in a mutant of *Arabidopsis thaliana*, squint, that appears not to undergo phase changes and appears adult-like throughout its growth cycle, compared with wild type were identified, compared to the Ceres full length cDNA and genomic sequence databanks, and equivalent Ceres clones identified. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables reports the results of this analysis, indicating those Ceres clones which are up or down regulated over controls, thereby indicating the Ceres clones which represent phase responsive genes. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: Sqn (relating to SMD 7133, SMD 7137)). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Phase responsive genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### Phase Responsive Genes Identified By Cluster Analyses Of Differential Expression

##### Phase Responsive Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of phase responsive genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID Sqn (relating to SMD 7133, SMD 7137) of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

##### Phase Responsive Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of phase responsive genes. A group in the MA\_clust is considered a phase responsive pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

#### Phase Responsive Genes Identified By Amino Acid Sequence Similarity

Phase responsive genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis phase responsive genes. Groups of phase responsive genes are identified in the Protein Grouping table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a phase responsive pathway or network is a group of proteins that also exhibits Phase responsive functions/utilities.

Further, promoters of phase responsive genes, as described in Reference tables, for example, are useful to modulate transcription that is induced by phase or any of the following phenotypes or biological activities below. Further, any desired sequence can be transcribed in similar temporal, tissue, or environmentally specific patterns as the phase responsive genes when the desired sequence is operably linked to a promoter of a phase responsive gene.

#### a) USE OF PHASE RESPONSIVE GENES TO MODULATE PHENOTYPES

Phase responsive genes and gene products are useful to or modulate one or more of the following phenotypes:

- Timing Phenotypes
- Dormancy
- Germination
- Cotyledon opening
- First leaves
- Juvenile to adult transition
- Bolting
- Flowering

- Pollination
- Fertilization
- Seed development
- Seed set
- Fruit Drop
- Senescence
- Epinasty
- Biomass
- Fresh and Dry Weight during any time in plant life, such as maturation
- Number of Flowers, Seeds, Branches, and/or Leaves
- Seed Yield, including Number, Size, Weight, and/or Harvest Index
- Fruit Yield, including Number, Size, Weight, and/or Harvest Index
- Plant Development
- Time to Fruit Maturity
- Cell Wall Strengthening and Reinforcement
- Stress Tolerance
- Drought tolerance
- Flooding tolerance
- UV tolerance

To regulate any of the phenotype(s) above, activities of one or more of the phase responsive genes or gene products can be modulated and the plants can be tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be screened for variants as in Anderson et al. (1997) *Plant Cell* 9: 1727-1743; Heintzen et al. (1997) *Proc. Natl. Acad. Sci. USA* 94: 8515-20; Schaffer et al. (1998) *Cell* 93:1219-1229; Somers et al. (1998) *Development* 125: 485-494; Somers et al. (1998) *Science* 282: 1488-1490; Wang and Tobin (1998) *Cell* 93: 1207-1217; Zhong et al. (1998) *Plant Cell* 10: 2005-2017; Sugano et al. (1998) *Proc. Natl. Acad. Sci. USA* 95: 11020-11025; Dowson-Day and Millar (1999) *Plant J* 17: 63-71; Green and Tobin (1999) *Proc. Natl. Acad. Sci. USA* 96: 4176-419; Staiger and Apel (1999) *Mol. Gen. Genet.* 261: 811-819; Strayer and Kay (1999) *Curr. Opin. Plant Biol.* 2:114-120; Strayer et. al. (2000)

*Science* 289:768-771; Kreps et al. (2000) *J Biol Rhythms* (2000) 15:208-217; Nelson et al. (2000) *Cell* 101:331-340; Somers et al. (2000) *Cell* 101:319-329.

b) USE OF PHASE RESPONSIVE GENES TO MODULATE  
BIOCHEMICAL ACTIVITIES

5

he activities of one or more of the phase responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities are documented and can be measured according to the citations above and included in the table below:

Process	Biochemical Or Metabolic Activities And/Or Pathways	Citations including assays
Germination And Seedling Development	Cold, Light And Water Modulated Signal Transduction Pathways, Receptors, Kinases, PAS Domain Proteins	Bognar et al. (1999) <i>Proc. Natl. Acad. Sci. USA</i> 96:14652-14657; Sugano et al (1999) <i>Proc. Natl. Acad. Sci. USA</i> 96:12362-12366; Dowson-Day and Millar (1999) <i>Plant J</i> 17: 63-71; Somers et al. (2000) <i>Cell</i> 101:319-329; Zhong et al. (1998) <i>Plant Cell</i> 10: 2005-2017
Growth Transitions And Flowering	Cold And Light Modulated Signal Transduction Pathways, Receptors, Kinases, PAS Domain Proteins	Nelson et al. (2000) <i>Cell</i> 101:331-340; Fowler et al. (1999) <i>EMBO J.</i> 18:4679-4688
Tuber Formation	Cold And Light Modulated Signal Transduction Pathways	Yanovsky et al. (2000) <i>Plant J.</i> 23: 223-232

Process	Biochemical Or Metabolic Activities And/Or Pathways	Citations including assays
<u>METABOLISM</u>		
Lipid Metabolism	Membrane Lipid Synthesis Including Omega-3 Fatty Acid Desaturase, Lipases, Lipid Transfer Proteins	Bradley and Reddy (1997) <i>J. Bacteriol.</i> 179: 4407-4410; Martin, M et al. 1999 <i>Europe J. Biochem</i> 262: 283-290
Sugar Metabolism	Glycosylhydrolases, Glycosyltransferases, Amylases, Sucrose Synthase, CAB, Rubisco, Light Signal Transduction	Liu et al. (1996) <i>Plant Physiol.</i> 112:43-51; Millar and Kay (1996) <i>Proc Natl Acad Sci U S A</i> 93:15491-15496; Wang et al. (1997) <i>Plant Cell</i> 9:491-507; Shinohara et al (1999) <i>J. Biol. Chem.</i> 273: 446-452
Nitrogen Metabolism	Aminotransferases, Arginase, Proteases And Vegetative Storage Proteins, Aromatic Amino Acid Synthesis	Bradley and Reddy (1997) <i>J. Bacteriol.</i> 179: 4407-4410
Photorespiration	Mitochondrial, Chloroplast And Peroxisomal Photorespiratory Enzymes, Serine Hydroxymethyl Transferases, Catalase	Zhong and McClung (1996) <i>Mol. Gen. Genet.</i> 251:196-203; McClung (1997) <i>Free. Radic. Biol. Med.</i> 23:489-496; McClung et al. (2000) <i>Plant Physiol.</i> 123:381-392
Responses To Environmental Stress	Expression Of Genes Involved In Responses To Drought, Salt, UV	McClung (1997) <i>Free Radic Biol Med</i> 23:489-496; Shi et al. (2000) <i>Proc. Natl. Acad. Sci. USA</i> 97:6896-6901



Other biological activities that can be modulated by the phase responsive genes and their products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table.

Phase responsive genes are characteristically differentially transcribed in response to maturity of the cell, organ or tissue which depends on a timing mechanism, which is internal to an organism or cell. The Intensity Table reports the changes in transcript levels of various phase responsive genes in a plant.

The data from this experiment reveal a number of types of phase responsive genes and gene products. Profiles of some classes of phase responsive genes are shown in the table below with examples of which associated biological activities are modulated when the activities of one or more such genes vary in plants.

Transcript Levels	Type Of Genes	Physiological Consequences	Examples Of Biochemical Activity
Up Regulated Transcripts	<p>Responders To mutation that confers adult like phase</p> <p>Genes induced in adult-like phase</p>	<ul style="list-style-type: none"> <li>• Adult phase adoption</li> <li>• Metabolisms Affected By phase change</li> <li>• Synthesis Of Secondary Metabolites And/Or Proteins</li> <li>• Modulation Of Phase Response Transduction Pathways</li> <li>• Specific Gene Transcription Initiation</li> </ul>	<ul style="list-style-type: none"> <li>• Metabolic Enzymes</li> <li>• Change In Cell Membrane Structure And Potential</li> <li>• Kinases And Phosphatases</li> <li>• Transcription Activators</li> <li>• Change In Chromatin Structure And/Or Localized DNA Topology</li> </ul>
Down-Regulated Transcripts	<p>Responders To mutation that confers adult phase</p> <p>Genes repressed in adult-like phase</p> <p>Genes With Discontinued</p>	<ul style="list-style-type: none"> <li>• Negative Regulation of adult phase pathways</li> <li>• Changes In Pathways And Processes Operating In Cells</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> <li>• Change In Protein Structure By Phosphorylation (Kinases) Or Dephosphorylation (Phosphatases)</li> <li>• Change In Chromatin Structure And/Or</li> </ul>

Transcript Levels	Type Of Genes	Physiological Consequences	Examples Of Biochemical Activity
	Expression Or Unstable mRNA in adult-like phase	<ul style="list-style-type: none"> <li>Changes In Metabolic pathways other than phase specific pathways</li> </ul>	DNA Topology <ul style="list-style-type: none"> <li>Stability Factors For Protein Synthesis And Degradation</li> <li>Metabolic Enzymes</li> </ul>

#### Use of Promoters of Phase Responsive Genes

Promoters of phase responsive genes are useful for transcription of any desired polynucleotide or plant or non-plant origin. Further, any desired sequence can be transcribed in a similar temporal, tissue, or environmentally specific patterns as the phase responsive genes where the desired sequence is operably linked to a promoter of a phase responsive gene. The protein product of such a polynucleotide is usually synthesized in the same cells, in response to the same stimuli as the protein product of the gene from which the promoter was derived. Such promoter are also useful to produce antisense mRNAs to down-regulate the product of proteins, or to produce sense mRNAs to down-regulate mRNAs via sense suppression.

II. HORMONE RESPONSIVE GENES, GENE COMPONENTS AND PRODUCTS

II.A. ABSCISSIC ACID RESPONSIVE GENES, GENE COMPONENTS AND  
PRODUCTS

Plant hormones are naturally occurring substances, effective in very small amounts, which act as signals to stimulate or inhibit growth or regulate developmental processes in plants. Abscissic acid (ABA) is a ubiquitous hormone in vascular plants that has been detected in every major organ or living tissue from the root to the apical bud. The major physiological responses affected by ABA are dormancy, stress stomatal closure, water uptake, abscission and senescence. In contrast to auxins, cytokinins and gibberellins, which are principally growth promoters, ABA primarily acts as an inhibitor of growth and metabolic processes.

Changes in ABA concentration internally or in the surrounding environment in contact with a plant results in modulation of many genes and gene products. Examples of such ABA responsive genes and gene products are shown in the Reference, Sequence, Protein Group, Protein Group Matrix tables, MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff, and MA\_clust tables. These genes and/or products are responsible for effects on traits such as plant vigor and seed yield. They were discovered and characterized from a much larger set of genes by experiments designed to find genes whose mRNA products changed in concentration in response to application of ABA to plants.

While ABA responsive polynucleotides and gene products can act alone, combinations of these polynucleotides also affect growth and development. Useful combinations include different ABA responsive polynucleotides and/or gene products that have similar transcription profiles or similar biological activities, and members of the same or similar biochemical pathways. Whole pathways or segments of pathways are controlled by transcription factor proteins and proteins controlling the activity of signal transduction pathways. Therefore, manipulation of such protein levels is especially useful for altering phenotypes and biochemical activities of plants. In addition, the combination of an ABA responsive polynucleotide and/or gene product with another environmentally responsive polynucleotide is also useful because of the interactions that exist between hormone regulated pathways, stress and defence induced pathways, nutritional pathways and development. Here, in addition to polynucleotides having

similar transcription profiles and/or biological activities, useful combinations include polynucleotides that may have different transcription profiles but which participate in common or overlapping pathways.

Such ABA responsive genes and gene products can function to either increase or dampen the above phenotypes or activities either in response to changes in ABA concentration or in the absence of ABA fluctuations. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108560, 108561, 108513, 108597). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

ABA genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### ABA Genes Identified By Cluster Analyses Of Differential Expression

##### ABA Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of ABA genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108560, 108561, 108513, 108597 of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

##### ABA Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of ABA genes. A group in the MA\_clust is considered a ABA pathway or network if the group comprises a cDNA ID that also appears in

Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

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#### ABA Genes Identified By Amino Acid Sequence Similarity

ABA genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis ABA genes. Groups of ABA genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a ABA pathway or network is a group of proteins that also exhibits ABA functions/utilities.

Further, promoters of ABA responsive genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by ABA or any of the following phenotypes or biological activities below.

#### II.A.1. USE OF ABSCISSIC ACID RESPONSIVE GENES TO MODULATE PHENOTYPES

ABA responsive genes and gene products are useful to or modulate one or more of the following phenotypes:

- Development
  - Cell Growth
    - Promotion Of Leaf Cell Elongation
  - Fruit Development
    - Fruit Drop
    - Inhibition Of Parthenocarp And Ovary Growth
  - Seed Development
    - Maturation Of Zygotic And Somatic Embryos
    - Embryo Development
    - Seed Development And Maturation
    - Acquisition Of Desiccation Tolerance

- Dormancy
  - Control Rate And Timing Of Germination
  - Prolongation Of Seed Storage And Viability
  - Inhibition Of Hydrolytic Enzyme Synthesis
- Growth
  - Roots
    - Inhibition Of Root Elongation Under Low Water Potential
  - Stems
  - Buds
    - Promotion Of Dormancy
    - Lateral/Axillary Bud Formation
  - Leaves
  - Inhibition Of ABA-Induced Growth And Elongation
- Biomass
  - Fresh And Dry Weight During Any Time In Plant Life, Such As Maturation;
  - Number, Size, And Weight Of
    - Flowers;
    - Seeds;
- Senescence
  - Abscission
  - Leaf Fall
  - Flower Longevity
- Differentiation
  - Plastid/Chloroplast Differentiation
  - Regulation Of Sterility
- Stress Responses
  - Mediation Of Response To Desiccation, Drought, Salt And Cold

To regulate any of the phenotype(s) above, activities of one or more of the ABA responsive genes or gene products can be modulated in an organism and tested by screening for

the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266) and/or screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and visually inspected for the desired phenotype or metabolically and/or functionally assayed according to Koorneef and Karssen (1994, Seed dormancy and germination, In: Arabidopsis, Cold Spring harbor Lab. Press, pp 314-334), Cramer et al (1998, J. Exptl. Botany 49:191-198), and White and Rivin (2000, Plant Physiol 122: 1089-97). Phillips et al. (1997) EMBO J 16: 4489-96; Nambara et al (1995) Development 121: 629-636; Hays et al (1999) Plant Physiol. 119: 1065-72; Filonova et al (2000) J Exptl Botany 51: 249-64; White et al (2000) Plant Physiol. 122: 1081-88; and Visser et al. (1998) Plant Mol Biol 37: 131-40; Rohde et al. (2000) Plant Cell 12:35-52; and Cramer et al. (1998) J. experimental Botany. 49: 191-198.

#### II.A.2. USE OF ABSCISSIC ACID RESPONSIVE GENES TO MODULATE BIOCHEMICAL ACTIVITIES

The activities of one or more of the ABA responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Growth , Differentiation And Development	<ul style="list-style-type: none"> <li>Farnesylation</li> <li>Nitrogen Metabolism</li> </ul>	Pei Et Al (1998) Science 282: 287-290; Cutler Et Al. (1996) Science 273: 1239 Goupil Et Al (1998) J Exptl Botany 49:1855-62
Water Conservation And Resistance To Drought And Other Related Stresses	<ul style="list-style-type: none"> <li>Stomatal Development And Physiology</li> </ul>	Allen Et Al. (1999) Plant Cell 11: 1785-1798 Li Et Al. 2000 Science 287: 300-303 Burnett Et Al 2000. J. Exptl Botany 51: 197-205

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	<ul style="list-style-type: none"> <li>• Stress Response Pathways</li> <li>• Inhibition Of Ethylene Production Under Low Water Potential</li> <li>• Proline And Other Osmolite Synthesis And Degradation</li> </ul>	<p>Raschke (1987) In: Stomatal Function Zeiger Et Al. Eds., 253-279</p> <p>Bush And Pages (1998) Plant Mol. Biol. 37: 425-35</p> <p>Spollen Et Al (2000) Plant Physiol. 122:967-976</p> <p>Hare Et Al. (1998) Plant, Cell And Environment 21:535-553; Hare Et Al. (1999) J. Exptl. Botany 50:413-434</p>
	<ul style="list-style-type: none"> <li>• Plasmalemma And Tonoplast Ion Channel Changes</li> <li>• Ca<sup>2+</sup> Accumulation</li> <li>• K<sup>+</sup> Efflux</li> <li>• Activation Of Kinases And Phosphatases</li> </ul>	<p>Macrobbie (1998) Philos Trans R Soc Lond B Biol Sci 353: 1475-88; Li Et Al (2000) Science 287:300-303; Barkla Et Al. (1999) Plant Physiol. 120:811-819</p> <p>Lacombe Et Al. (2000) Plant Cell 12: 837-51; Wang Et Al. (1998) Plant Physiol 118:1421-1429; Shi Et Al. (1999) Plant Cell 11: 2393-2406</p> <p>Gaymard Et Al. (1998) Cell 94:647-655</p> <p>Jonak Et Al. (1996) Proc. Natl. Acad. Sci 93: 11274-79; Sheen (1998) Proc. Natl. Acad. Sci. 95: 975-80; Allen Et Al. (1999) Plant Cell 11: 1785-98</p>



Other biological activities that can be modulated by the ABA responsive genes and gene products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table.

ABA responsive genes are characteristically differentially transcribed in response to fluctuating ABA levels or concentrations, whether internal or external to an organism or cell. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff reports the changes in transcript levels of various ABA responsive genes in entire seedlings at 1 and 6 hours after a plant was sprayed with a Hoagland's solution enriched with ABA as compared to seedlings sprayed with Hoagland's solution only.

The data from this time course can be used to identify a number of types of ABA responsive genes and gene products, including "early responders," and "delayed ABA responders", "early responder repressors" and "delayed repressors". Profiles of these different ABA responsive genes are shown in the Table below together with examples of the kinds of associated biological activities.

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
Up Regulated Transcripts (Level At 1 Hr $\cong$ 6 Hr) or (Level At 1 Hr > 6 Hr)	Early Responders To ABA	ABA Perception ABA Uptake  Modulation Of ABA Response Transduction Pathways Specific Gene Transcription Initiation	Transcription Factors Transporters Change In Cell Membrane Structure Kinases And Phosphatases  Transcription Activators Change In Chromatin Structure And/Or Localized DNA Topology
Up Regulated Transcripts (Level At 1 Hr < 6 Hr)	Delayed Responders	Maintenance Of Response To ABA Maintenance Of Seed Dormancy, Stress Stomatal Closure, Water Uptake Control, Abscission And Senescence Control Pathways	Transcription Factors Specific Factors (Initiation And Elongation) For Protein Synthesis Maintenance Of Mrna Stability Maintenance Of Protein Stability Maintenance Of Protein-Protein Interaction
Down-Regulated Transcripts (Level At 1 Hr $\cong$ 6 Hr) or (Level At 6 Hr > 1 Hr)	Early Responder Repressors Of ABA State Of Metabolism	Negative Regulation Of ABA Pathways Released  Changes In Pathways	Transcription Factors Change In Protein Structure By Phosphorylation (Kinases) Or Dephosphorylation

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TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
Hr)	Genes With Discontinued Expression Or UnsTable mRNA In Presence Of ABA	And Processes Operating In Cells	(Phosphatases) Change In Chromatin Structure And/Or DNA Topology
Down-Regulated Transcripts (Level At 1 Hr > 6 Hr)	Delayed Repressors Of ABA State Of Metabolism  Genes With Discontinued Expression Or UnsTable mRNA In Presence Of ABA	Negative Regulation Of ABA Pathways Released  Maintenance Of Pathways Released From Repression  Changes In Pathways And Processes Operating In Cells	Transcription Factors Kinases And Phosphatases Stability Of Factors For Protein Synthesis And Degradation

#### Use of Promoters of ABA responsive Genes

Promoters of ABA responsive genes are useful for transcription of any desired polynucleotide or plant or non-plant origin. Further, any desired sequence can be transcribed in a similar temporal, tissue, or environmentally specific patterns as the ABA responsive genes where the desired sequence is operably linked to a promoter of a ABA responsive gene. The protein product of such a polynucleotide is usually synthesized in the same cells, in response to the same stimuli as the protein product of the gene from which the promoter was derived. Such promoter are also useful to produce antisense mRNAs to down-regulate the product of proteins, or to produce sense mRNAs to down-regulate mRNAs via sense suppression.

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II.B. AUXIN RESPONSIVE GENES, GENE COMPONENTS AND PRODUCTS

Plant hormones are naturally occurring substances, effective in very small amounts that stimulate or inhibit growth or regulate developmental processes in plants. One of the plant hormones is indole-3-acetic acid (IAA), often referred to as Auxin.

5 Changes in Auxin concentration in the surrounding environment in contact with a plant or in a plant results in modulation of the activities of many genes and hence levels of gene products. Examples of such Auxin responsive genes and their products are shown in the Reference and Sequence Tables. These genes and/or products are responsible for effects on traits such as plant vigor and seed yield. The genes were discovered and characterized from a  
10 much larger set by experiments designed to find genes whose mRNA products changed in response to application of Auxin to plants.

15 Manipulation of one or more Auxin responsive gene activities are useful to modulate the biological activities and/or phenotypes listed below. Auxin response genes and gene products can act alone or in combination. Useful combinations include Auxin response genes and/or gene products with similar transcription profiles, similar biological activities, or members of the same or functionally related biochemical pathways. Whole pathways or segments of pathways are controlled by transcription factor proteins and proteins controlling the activity of signal transduction pathways. Therefore, manipulation of the levels of such proteins is especially useful for altering phenotypes and biochemical activities of plants. The MA\_diff and/or AFLP\_diff  
20 MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108564, 108565, 108516, 108554, 108466, 107886, 107891, SMD 3743, and NAA (relating to SMD 3749, SMD 6338, SMD 6339)). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the  
25 Example section below.

NAA genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

30 NAA Genes Identified By Cluster Analyses Of Differential Expression

NAA Genes Identified By Correlation To Genes That Are Differentially

Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of NAA genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108564, 108565, 108516, 108554, 108466, 107886, 107891, SMD 3743, and NAA (relating to SMD 3749, SMD 6338, SMD 6339) of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

NAA Genes Identified By Correlation To Genes That Cause Physiological

Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of NAA genes. A group in the MA\_clust is considered a NAA pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

NAA Genes Identified By Amino Acid Sequence Similarity

NAA genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis NAA genes. Groups of NAA genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a NAA pathway or network is a group of proteins that also exhibits NAA functions/utilities.

Such Auxin responsive genes and gene products can function to either increase or dampen the above phenotypes or activities either in response to changes in Auxin concentration or in the absence of Auxin fluctuations. Further, promoters of Auxin responsive genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by auxin or any of the following phenotypes or biological activities below.

II.B.1. USE OF AUXIN RESPONSIVE GENES, GENE COMPONENTS AND  
PRODUCTS TO MODULATE PHENOTYPES

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Auxin Responsive Genes And Gene Products Are Useful To Or Modulate One Or  
More Of The Following Phenotypes:

- Growth
- Apical Dominance
- Vascular Growth
- Roots
- Inhibition Of Primary Root Elongation
- Increased Lateral Root Formation
- Stems
- Lateral Buds
- Lateral Branching
- Reduction Of Branching
- For High Density Growth Per Acre
- For Increased Wood Production
- Lateral Organ Initiation And/Or Positioning In Apical Meristem,
- Organ Formation, For Example, Fruit Number In Tomatoes
- Leaves
- Height/Stature, E.G., Taller Crops Or Increase Wood Production
- Regeneration And Differentiation Of Cultured Cells Or Plantlets
- Biomass
- Fresh And Dry Weight During Any Time In Plant Life, Such As Maturation;
- Number Of Flowers;
- Number Of Seeds;
- Number Of Branches;
- Number Of Leaves;
- Starch Content

- Seed Yield, Including Number, Size, Weight, Harvest Index, Starch Content
- Fruit Yield, Number, Size, Weight, Harvest Index, Starch Content
- Development
- Orienting Cell Growth,
- Establishment And Maintenance Of Plant Axis
- Apical Dominance
- Cell Plate Placement
- Polarised Growth, Initiation And/Or Development, Of Embryos Morphogenic Progression, E.G., From Early Radial To Late Axialized Torpedo Stages
- Differentiation Of Cells Into Morphologically Different Cell Layers
- Cotyledon Separation
- Fruit Development
- Abscission, Leading To Modulation Of Fruit Drop
- Parthenocarp, Seedless Crops Resulting From Lack Of Seed Set
- Vascularization, E.G. Hypocotyl And Cotyledon Tissues
- Genetic Control Of Vascular Patterning And Influences Its Maturation;
- Specification Of The Sites Where Vascular Differentiation Will Occur;
- Determination Of The Direction And Extent Of Vascular Tissue Formation
- Maintenance Of The Continuity Of Vascular Development With Plant Growth
- Tropic Responses
- Gravitropic Responses, E.G. Affecting Roots And Shoots
- Modulation Of Phototropic Sensitivity, E.G. Increase Growth Under A Reduced Light Spectrum

Further, any desired sequence can be transcribed in similar temporal, tissue, or environmentally specific patterns as the Auxin responsive genes when the desired sequence is operably linked to a promoter of an Auxin responsive gene.

To modulate any of the phenotype(s) above, activities of one or more of the Auxin response genes or gene products can be modulated and the plants can be tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be



screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and assayed, for example, in accordance with Bechtold and Pelletier (1998). Methods Mol. Biol. 82: 259-266; Clough and Bent (1998). 16: 735-743; Krysan et al. (1999). Plant Cell 11:2283-2290.

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II.B.2. USE OF AUXIN RESPONSIVE GENES, GENE COMPONENTS AND  
PRODUCTS TO BIOCHEMICAL ACTIVITIES:

The activities of one or more of the Auxin responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities are documented and can be measured according to the citations included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Cell Growth and Differentiation	Protein Ubiquitination	Gray et al. (1999) Genes and Develop, 13:1678-1691 Bechtold and Pelletier (1998). Methods. Mol. Biol. 82:259-266
	Cell Wall loosening and Expansion	Catala et al. (2000). Plant Physiol. 122:527-534. Cosgrove, D. (1993). New Phytol. 124:1-23.

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PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Auxin/Cytokinin Ratio	Changing Auxin and/or cytokinin synthesis and/or turnover	Chen et al. (1988). Plant Physiol. 86:822-825 Tam et al. (2000). Plant Physiol. 123:589-595 Bartel and Fink. (1995). Science 268:1745-1748. Prinsen et al. (1995). Quantifying phytohormones in transformed plants. In: Methods in Molecular Biology. 44:245-262.
Auxin Transport	Channeling of polar Auxin Transport	Reed et al. (1998). Plant Physiol. 118:1369-1378. Estelle, M. (1998). Plant Cell 10:1775-1778
	Auxin Efflux Between Cells	Reed et al. (1998). Plant Physiol. 118:1369-1378. Marchant et al. (1999). EMBO J. 18:2066-2073.
	Auxin Influx In and Out of a Cell	Reed et al. (1998). Plant Physiol. 118:1369-1378. Marchant et al. (1999). EMBO J. 18:2066-2073.
	Electrogenic Proton Symport of Auxin	Young et al. (1999). Biochim Biophys Acta. 1415(2):306-22
Signal Transduction	K <sup>+</sup> Accumulation	Philippa et al. (1999). Proc. Natl. Acad. Sci. 96:12186-12191

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	Permeability of Cell Membranes	Marchant et al. (1999). EMBO J. 18:2066-2073.
	Guanine-Nucleotide Exchange	Steinmann et al. (1999). Science 286:316-318. Peyroche et al. (1996). Nature 384:479-481.
	Protein Phosphorylation	Christensen et al. (2000). Cell 100:469-478. Hirt (2000). Proc. Natl. Acad Sci. 97:2405-2407.
	Interaction with Ethylene mode of action	Madlung et al. (1999). Plant Physiol. 120:897-906. Xu et al. (1998). Plant Physiol. 118:867-874.
Protein Turnover	Localization of Polypeptides with the basal End of Cells	Grebe et al. (2000). Plant Cell. 12:343-356

Other biological activities that can be modulated to by the Auxin responsive genes and their products are listed in the Reference Tables. Assays for detecting such biological activities are described in the Domain section of the Reference Tables.

Auxin responsive genes are characteristically differentially transcribed in response to fluctuating Auxin levels or concentrations, whether internal or external to an organism or cell. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff (s) report(s) the changes in transcript levels of various Auxin responsive genes in the aerial parts of a seedling at 1 and 6 hours after the seedling was sprayed with an solution enriched with Auxin as compared to aerial parts of a seedling sprayed with water.

The data from this time course can be used to identify a number of types of Auxin responsive genes and gene products, including "early responders," and "delayed

responders." Profiles of these different classes of Auxin responsive genes are shown in the Table below together with examples of the kinds of associated biological activities.

TRANSCRIPT LEVEL	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY OF GENE PRODUCTS
Upregulated transcripts (level at 1 hr $\leq$ 6hours) (level at 1 hr>6 hours)	Early responders to Auxin	<ul style="list-style-type: none"> <li>• Auxin perception</li> <li>• Auxin Uptake/transport</li> <li>• Modulation of Auxin response transduction pathways</li> <li>• Initiating transcription of specific gene(s)</li> <li>• Modification of cell walls</li> <li>• Modification of cell structures</li> <li>• Modification of metabolism</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription factors</li> <li>• Transporters; channeling of polar Auxin transport</li> <li>• Kinases and phosphatases; protein ubiquitination; guanine nucleotide exchange; changing Auxin and/or cytokinin synthesis and/or turnover; interaction with ethylene mode of action</li> <li>• Auxin metabolic pathways</li> <li>• Change in chromatin structure and/or DNA topology</li> <li>• Transcriptional activators</li> <li>• Change in activity of protein-protein interactions</li> <li>• Cell wall and cell growth promoting pathways</li> <li>• Change in activity of cytoskeletal proteins modulating cell structure</li> <li>• Metabolic enzymes</li> <li>• Coordination and control of central carbon and Auxin metabolism</li> </ul>

TRANSCRIPT LEVEL	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY OF GENE PRODUCTS
Upregulated transcripts (level at 1 hr <6 hr)	"Delayed" Responders	<ul style="list-style-type: none"> <li>• Completion and/or Maintenance of Auxin response</li> <li>• Initiating transcription of specific gene(s)</li> <li>• Modification of cell walls</li> <li>• Modification of cell structures</li> <li>• Modification of metabolism</li> </ul>	<p>Transcription factors</p> <p>Changes in membrane protein, membrane channel and/or transporter protein activity</p> <ul style="list-style-type: none"> <li>- Change in chromatin structure and/or DNA topology</li> <li>- Transcriptional activators</li> <li>- Change in activity of protein-protein interactions</li> <li>- Cell wall proteins</li> <li>- Change(s) in activity of cytoskeletal proteins modulating cell structure</li> <li>- Coordination and control of central carbon and Auxin metabolism</li> <li>-metabolic enzymes</li> </ul>

TRANSCRIPT LEVEL	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY OF GENE PRODUCTS
Downregulated transcripts (level at 1 hour $\cong$ 6 hours) (level at 1 hour > 6 hours)	Early repressor responders to Auxin  Genes for pathways diminished in presence of Auxin	<ul style="list-style-type: none"> <li>• Repression of Auxin induced proteins released</li> <li>• Reorientation of metabolism in certain cells</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription factors</li> <li>• Changes in activity of cytoskeletal proteins modulating cell structure</li> <li>• Changes in chromatin structure and/or DNA topology</li> <li>• Changes in protein structure and/or function by phosphorylation (kinases) and/or dephosphorylation (phosphatases)</li> <li>• Stability of factors for protein translation</li> <li>• Changes in cell membrane structure</li> <li>• Changes in chromatin and/or localized DNA topology</li> <li>• Changes in protein-protein interaction</li> <li>• Metabolic enzymes</li> </ul>

TRANSCRIPT LEVEL	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY OF GENE PRODUCTS
Down-regulated transcripts (level at 1 hour < 6 hours)	<p>"Delayed" repressor responders to Auxin</p> <p>Genes for pathways diminished in presence of Auxin</p>	<ul style="list-style-type: none"> <li>Maintenance of Auxin stimulated state(s) in certain cells</li> <li>Reorientation of metabolism in certain cells</li> </ul>	<ul style="list-style-type: none"> <li>Transcription factors</li> <li>Change in activity of cytoskeletal proteins modulating cell structure</li> <li>Changes in chromatin structure and/or DNA topology</li> <li>Changes in protein structure and/or function by phosphorylation (kinases) and/or dephosphorylation (phosphatases)</li> <li>Stability of factors for protein translation</li> <li>Changes in cell membrane structure</li> <li>Changes in chromatin and/or localized DNA topology</li> <li>Changes in protein-protein interaction</li> <li>Metabolic enzymes</li> </ul>

#### Use of Promoters of NAA responsive Genes

Promoters of NAA responsive genes are useful for transcription of any desired polynucleotide or plant or non-plant origin. Further, any desired sequence can be transcribed in a similar temporal, tissue, or environmentally specific patterns as the NAA responsive genes where the desired sequence is operably linked to a promoter of a NAA responsive gene. The protein product of such a polynucleotide is usually synthesized in the same cells, in response to the same stimuli as the protein product of the gene from which the promoter was derived. Such promoter are also useful to produce antisense mRNAs to down-regulate the product of proteins, or to produce sense mRNAs to down-regulate mRNAs via sense suppression.



II.C. BRASSINOSTEROID RESPONSIVE GENES, GENE COMPONENTS AND  
PRODUCTS:

Plant hormones are naturally occurring substances, effective in very small amounts, which act as signals to stimulate or inhibit growth or regulate developmental processes in plants.

5 Brassinosteroids (BRs) are the most recently discovered, and least studied, class of plant hormones. The major physiological response affected by BRs is the longitudinal growth of young tissue via cell elongation and possibly cell division. Consequently, disruptions in BR metabolism, perception and activity frequently result in a dwarf phenotype. In addition, because BRs are derived from the sterol metabolic pathway, any perturbations to the sterol pathway can affect the BR pathway. In the same way, perturbations in the BR pathway can have effects on the later part of the sterol pathway and thus the sterol composition of membranes.

10 Changes in BR concentration in the surrounding environment or in contact with a plant result in modulation of many genes and gene products. Examples of such BR responsive genes and gene products are shown in the Reference and Sequence Tables. These genes and/or products are responsible for effects on traits such as plant biomass and seed yield. These genes were discovered and characterized from a much larger set of genes by experiments designed to find genes whose mRNA abundance changed in response to application of BRs to plants.

15 While BR responsive polynucleotides and gene products can act alone, combinations of these polynucleotides also affect growth and development. Useful combinations include  
20 different BR responsive polynucleotides and/or gene products that have similar transcription profiles or similar biological activities, and members of the same or functionally related biochemical pathways. Whole pathways or segments of pathways are controlled by transcription factors and proteins controlling the activity of signal transduction pathways. Therefore, manipulation of such protein levels is especially useful for altering phenotypes and biochemical  
25 activities of plants. In addition, the combination of a BR responsive polynucleotide and/or gene product with another environmentally responsive polynucleotide is useful because of the interactions that exist between hormone regulated pathways, stress pathways, nutritional pathways and development. Here, in addition to polynucleotides having similar transcription profiles and/or biological activities, useful combinations include polynucleotides that may have  
30 different transcription profiles but which participate in common or overlapping pathways. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the

transcript levels of the experiment (see EXPT ID: 108580, 108581, 108557, 108478, 108479, 108480, 108481). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

5 BR genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### BR Genes Identified By Cluster Analyses Of Differential Expression

##### BR Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of BR genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108580, 108581, 108557, 108478, 108479, 108480, 108481 of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

##### BR Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of BR genes. A group in the MA\_clust is considered a BR pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

##### BR Genes Identified By Amino Acid Sequence Similarity

BR genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis BR genes. Groups of BR

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- Leaves
- Increase Biomass
- Flowers
- Increase Reproduction
- Biomass
- Fresh And Dry Weight During Any Time In Plant Life, Such As Maturation;
- Number Of Flowers;
- Number Of Seeds;
- Number Of Branches;
- Number Of Leaves;
- Starch Content
- Seed Yield, Including Number, Size, Weight, Harvest Index, Starch Content
- Fruit Yield, Number, Size, Weight, Harvest Index, Starch Content
- Development
- Morphogenesis
- Control Of Organ Size And Shape
- Development Of New Ornamentals
- Control Of Leaf Size And Shape
- Promotes Leaf Unrolling And Enlargement
- For Development Of New Leafy Ornamentals
- Seed Development
- Inhibition Of De-Etiolation
- Dormancy
- Accelerated Germination At Low Temperatures
- Root
- Gravitropism
- Senescence
- Promoted In Light Grown Plants
- Inhibiting Synthesis Or Perception Could Extend Life Span Of Desired Tissues/Organs
- Differentiation

- Vascularization
- Promotes Xylem Differentiation
- Increases Xylem Fiber Length
- Resistance Responses
- Increases Resistance To Pathogens
- Tropic Responses

#### Gravitropic Responses Affecting Roots

Further, any desired sequence can be transcribed in similar temporal, tissue, or environmentally specific patterns as the BR responsive genes when the desired sequence is operably linked to a promoter of a BR responsive gene.

To improve any of the desired phenotype(s) above, activities of one or more of the BR response genes or gene products can be modulated and the plants tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266, and/or screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50, visually inspected for the desired phenotype and metabolically and/or functionally assayed according to Choe et al. (1999, Plant Cell 11:207-21 and Plant Physiol 119: 897-907), Yamamoto et al. (1997, Plant Cell Physiol 38:980-3), Asami and Yshida (1999, Trends in Plant Sciences, 4:348-353) and Azpiroz et al. (1998, Plant Cell 10:219-230)

#### II.C.2. USE OF BRASSINOSTEROID RESPONSIVE GENES TO MODULATE BIOCHEMICAL ACTIVITIES

The activities of one or more of the BR responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological

activities are documented and can be measured according to the citations included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
BR Transport	<ul style="list-style-type: none"> <li>BR Efflux Between Cells</li> </ul>	B.Schulz and K. Feldmann, unpub. results
	<ul style="list-style-type: none"> <li>BR Influx In And Out Of A Cell</li> </ul>	B.Schulz and K. Feldmann, unpub. results
Signal Transduction	<ul style="list-style-type: none"> <li>Permeability Of Cell Membranes</li> </ul>	
	<ul style="list-style-type: none"> <li>Protein Phosphorylation</li> </ul>	
Metabolism	<ul style="list-style-type: none"> <li>Major Growth Coordinating Pathways</li> </ul>	

Other biological activities that can be modulated by the BR responsive genes and gene products are listed in the Reference Tables. Assays for detecting such biological activities are described in the Domain section of the Reference Tables.

BR responsive genes are differentially transcribed in response to fluctuating BR levels or concentrations, whether internal or external to an organism or cell. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s) report(s) the changes in transcript levels of various BR responsive genes in the aerial parts of a seedling at 1 and 6 hours after a plant was sprayed with a solution enriched with BR as compared to seedlings sprayed with water. The data from this time course can be used to identify a number of types of BR responsive genes and gene products, including "early responders," "delayed responders." Profiles of these different categories of BR responsive genes are shown in the Table below together with examples of the kinds of associated biological activities.

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
Up Regulated Transcripts (Level At 1 Hr $\approx$ 6 Hr) (Level At 1 Hr $>$ 6 Hr)	<ul style="list-style-type: none"> <li>• Early Responders To BR</li> </ul>	<ul style="list-style-type: none"> <li>• BR Perception</li> <li>• BR Transport</li> <li>• BR Biosynthesis Feedback</li> <li>• Modulation Of BR Response Transduction Pathways</li> <li>• Specific Gene Transcription Initiation</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> <li>• Receptors</li> <li>• Transporters</li> <li>• Change In Cell Membrane Structure</li> <li>• Feedback Regulated Biosynthetic Genes</li> <li>• Kinases And Phosphatases</li> <li>• 2<sup>nd</sup> Messengers, Eg., Calmodulin</li> <li>• Transcription Activators</li> <li>• Change In Chromatin Structure And/Or Localized DNA Topology</li> </ul>
Up Regulated Transcripts (Level At 1 Hr $<$ 6 Hr)	<ul style="list-style-type: none"> <li>• Delayed Responders</li> </ul>	<ul style="list-style-type: none"> <li>• Maintenance Of Response To Br</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> <li>• BR Biosynthetic Genes</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
		<ul style="list-style-type: none"> <li>Cell And Organ Elongation</li> <li>Gravitropism</li> </ul>	<ul style="list-style-type: none"> <li>Specific Factors (Initiation And Elongation) For Protein Synthesis</li> <li>Maintenance Of Mrna Stability</li> <li>Maintenance Of Protein Stability</li> <li>Maintenance Of Protein-Protein Interaction</li> <li>Cell Wall Elongation</li> </ul>
Down-Regulated Transcripts (Level At 1 Hr $\approx$ 6 Hr) (Level At 6 Hr $>$ 1 Hr)	Early Responder Repressors Of BR State Of Metabolism  Genes With Discontinued Expression Or UnsTable Mrna In Presence Of BR	<ul style="list-style-type: none"> <li>Negative Regulation Of BR Pathways Released</li> <li>Changes In Pathways And Processes Operating In Cells</li> </ul>	<ul style="list-style-type: none"> <li>Transcription Factors</li> <li>Change In Protein Structure By Phosphorylation (Kinases) Or Dephosphoryaltion (Phosphatases)</li> <li>Change In Chromatin Structure And/Or DNA Topology</li> </ul>
Down-Regulated	<ul style="list-style-type: none"> <li>Delayed</li> </ul>	<ul style="list-style-type: none"> <li>Negative</li> </ul>	<ul style="list-style-type: none"> <li>Transcription</li> </ul>



TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
Transcripts (Level At 1 Hr > 6 Hr)	Repressors Of BR State Of Metabolism  • Genes With Discontinued Expression Or UnsTable Mrna In Presence Of BR	Regulation Of BR Pathways Released  • Maintenance Of Pathways Released From Repression  • Changes In Pathways And Processes Operating In Cells	Factors • Kinases And Phosphatases • Stability Of Factors For Protein Synthesis And Degradation

#### Use of Promoters of BR responsive Genes

Promoters of BR responsive genes are useful for transcription of any desired polynucleotide or plant or non-plant origin. Further, any desired sequence can be transcribed in a similar temporal, tissue, or environmentally specific patterns as the BR responsive genes where the desired sequence is operably linked to a promoter of a BR responsive gene. The protein product of such a polynucleotide is usually synthesized in the same cells, in response to the same stimuli as the protein product of the gene from which the promoter was derived. Such promoter are also useful to produce antisense mRNAs to down-regulate the product of proteins, or to produce sense mRNAs to down-regulate mRNAs via sense suppression.

II.D. CYTOKININ RESPONSIVE GENES, GENE COMPONENTS AND PRODUCTS

Plant hormones are naturally occurring substances, effective in very small amounts, which act as signals to stimulate or inhibit growth or regulate developmental processes in plants. Cytokinins (BA) are a group of hormones that are best known for their stimulatory effect on cell division, although they also participate in many other processes and pathways. All naturally occurring BAs are aminopurine derivatives, while nearly all synthetic compounds with BA activity are 6-substituted aminopurine derivatives. One of the most common synthetic BAs used in agriculture is benzylaminopurine (BAP).

Changes in BA concentration in the surrounding environment or in contact with a plant results in modulation of many genes and gene products. Examples of such BA responsive genes and gene products are shown in the Reference, Sequence, Protein Group, Protein Group Matrix tables, MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff and MA\_clust. These genes and/or products are responsible for effects on traits such as plant vigor and seed yield. They were discovered and characterized from a much larger set by experiments designed to find genes whose mRNA products changed in response to application of BA to plants.

While cytokinin responsive polynucleotides and gene products can act alone, combinations of these polynucleotides also affect growth and development. Useful combinations include different BA responsive polynucleotides and/or gene products that have similar transcription profiles or similar biological activities, and members of the same or functionally related biochemical pathways. Whole pathways or segments of pathways are controlled by transcription factor proteins and proteins controlling the activity of signal transduction pathways. Therefore, manipulation of such protein levels is especially useful for altering phenotypes and biochemical activities of plants. In addition, the combination of a BA responsive polynucleotide and/or gene product with another environmentally responsive polynucleotide is also useful because of the interactions that exist between hormone regulated pathways, stress pathways, nutritional pathways and development. Here, in addition to polynucleotides having similar transcription profiles and/or biological activities, useful combinations include polynucleotides that may have different transcription profiles but which participate in common or overlapping pathways. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108566, 108567, 108517).

For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

BA genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### BA Genes Identified By Cluster Analyses Of Differential Expression

##### BA Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of BA genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108566, 108567, 108517 of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

##### BA Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of BA genes. A group in the MA\_clust is considered a BA pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

##### BA Genes Identified By Amino Acid Sequence Similarity

BA genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis BA genes. Groups of BA genes are identified in the Protein Group table. In this table, any protein group that comprises a

peptide ID that corresponds to a cDNA ID member of a BA pathway or network is a group of proteins that also exhibits BA functions/utilities.

Such BA responsive genes and gene products can function to either increase or dampen the above phenotypes or activities either in response to changes in BA concentration or in the absence of BA fluctuations.

Further, promoters of BA responsive genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by BA or any of the following phenotypes or biological activities below.

#### II.D.1. USE OF BA-RESPONSIVE GENES TO MODULATE PHENOTYPES

BA responsive genes and gene products are useful to or modulate one or more of the following phenotypes:

- Growth
- Roots
  - Inhibition Of Elongation Of Root
- Stems
  - Inhibition Of Elongation Of Hypocotyl
- Lateral Buds
  - Promotion Of Outgrowth
    - For Rapid Production Of Multiple Shoots As A Source For Grafting
- Leaves
  - Development
    - Cell Growth
      - Expansion Of Cotyledon
      - Promotes Cell Enlargement
        - For Increased Yield From Leaf Crops
      - Chloroplast Development

- Delayed Degradation Of Chloroplasts
- For Increased Photosynthesis And Crop Yield
- Cell Division
  - For Increased Micropropagation
- Senescence
  - Delays
    - For Delayed Conversion From Photosynthesis To Salvage Programs In Leaves
    - For Increased Crop Yield
- Differentiation
  - Regulation Of Morphogenesis
    - For Manipulating Callus Growth And Shoot/Root Formation In Culture
- Maintenance Of Shoot Meristem
  - Increased Usable Wood Production
  - Reduced Tiller Number
    - For Denser Crop Planting Regimes
- Nutrient Metabolism
  - Effects On Seed Size
  - Effects On Rate Of Seed Set
    - For Increased Seed Yield
- Induction Of Ethylene Biosynthesis
  - Control Of Fruit Ripening
- Parthenocarpy
  - Control Of Sexual Reproduction
  - Production Of Seedless Fruits

To regulate any of the phenotype(s) above, activities of one or more of the BA responsive genes or gene products can be modulated and the plants tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998,

Methods. Mol. Biol. 82:259-266) and/or screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and visually inspected for the desired phenotype or molecularly or metabolically or functionally assayed according to Lohman et al (1994, Physil. Plant 92:322-328), Woolhouse (1983, In Agricultural Research- Strategies of Plant reproduction, Meudt, ed., 201-236), Medford et al. (1989, Plant Cell 1: 403-13), Vogel et al. (1998, Genetics 149:417-27), Ehnes and Roitsch (1997, Plant J 1: 539-48), Rotino et al. (1997, Nat. Biotechnol. 15: 1398-1401).

#### II.D.2. USE OF BA-RESPONSIVE GENES TO MODULATE BIOCHEMICAL ACTIVITIES

The activities of one or more of the BA responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Chloroplast Functioning	• Photosynthesis	Benkova et al (1999) Plant Physil 121: 245-252
Induction And Maintenance Of Cell Division	• Cell Cycle Phase Transition	Riou-Khamlichi et al. (1999) Science 283: 1541-44
Senescence	• Cell Death/Apoptosis	Lohman et al. (1994) Physiol Plant 92: 322-328
Signal Transduction	• Sensing Endogenous Stimuli To Trigger Growth And Shoot Formation	Kakimoto (1996) Science 274: 982-985

Other biological activities that can be modulated by the BA responsive genes and gene products are listed in the Reference tables. Assays for detecting such biological activities are described in the Domain section above.

BA responsive genes are characteristically differentially transcribed in response to fluctuating BA levels or concentrations, whether internal or external to an organism or cell. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table reports the changes in transcript levels of various BA responsive genes in the aerial parts of a seedling at 1 and 6 hours after a plant was sprayed with a Hoagland's solution enriched with BA as compared to seedlings sprayed with Hoagland's solution only.

The data from this time course can be used to identify a number of types of BA responsive genes and gene products, including "early responders," and "delayed responders." Profiles of these different BA responsive genes are shown in the Table below together with examples of the kinds of associated biological activities.

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENES	TYPE OF BIOLOGICAL ACTIVITY	EXAMPLES OF BIOCHEMICAL ACTIVITY
Up Regulated Transcripts (Level At 1h $\cong$ 6h) Or (Higher At 1h Than 6h)	<ul style="list-style-type: none"> <li>Early Responders To BA</li> </ul>	<ul style="list-style-type: none"> <li>- BA Perception</li> <li>-BA Uptake</li> <li>-Modulation Of BA Response</li> <li>Transduction Pathways</li> <li>-Specific Gene Transcription Initiation</li> <li>-Initiate And Coordinate Cell Division</li> <li>-Regulation Of Pathways To</li> </ul>	<ul style="list-style-type: none"> <li>-Transcription Factors</li> <li>-Transporters</li> <li>-Kinase, Receptor-Like Protein Kinase</li> <li>-Ovule-Specific Homeotic Protein, Secretory Pathway</li> <li>-Cell Division Control Protein, Cyclins, Cyclin-Dependent Protein Kinase (Cdk), Cell Cycle Phosphatases, Mitosis-Specific Chromosome Segregation Protein, Mitotic Phosphoprotein, Dna Replication Proteins, Helicase Telomerase, Centromere Protein, tRNA Synthase</li> <li>-Senescence-Associated Protein, Bifunctional Nuclease, Aba Pathway Genes, Ethylene Pathway Genes, Proteases, Nucleases, Pcd Genes</li> </ul>



GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENES	TYPE OF BIOLOGICAL ACTIVITY	EXAMPLES OF BIOCHEMICAL ACTIVITY
		Senescence	-Calvin Cycle, Chlorophyll A/B Binding Protein (Cab), Transketolase, Lipoxygenase, Chloroplast Rna Processing Protein, Chloroplast Envelope Membrane Protein.
		-Modulation Of Chloroplast Gene Expression And Photosynthesis	-Glutamate Synthase, Gogat, Asparagine Synthase, Catalase, Peroxidase
		-Modulation Of Photorespiration And Primary Nitrogen Assimilation In Leaves Expression	-Heat Shock Proteins, Gst -Fatty Acid Elongase- Like Protein, Very-Long- Chain Fatty Acid Condensing Enzyme, Coa Synthase
		-Stress Response	-Vicilin Storage Protein -Homeobox Domain Proteins
		-Wax Biosynthesis	-Mutase, Phosphoglycerate Mutase -Pectate Lyase, Ethylene Pathway Genes

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GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENES	TYPE OF BIOLOGICAL ACTIVITY	EXAMPLES OF BIOCHEMICAL ACTIVITY
		-Nutrient Metabolism -Embryogenesis  -Glycolysis, Gluconeogenesis  -Ripening	
Upregulated Transcripts (Higher At 6h Than 1h)	BA Late Responders	-BA Responsive Pathways  -Cell Wall Extension  -Organogenesis  -Modulate Activation Of Disease Defense Genes  -Modulate Responses To External Stimuli  -Osmotic Stress Tolerance	-Transfactors, Kinases, Phosphatases, LRR's, Dna Remodelling Proteins, Cu-Binding Proteins  -Expansins, Extensins, Proline Rich Proteins  -AP2 Domain Containing Proteins  -Transfactors Interacting With Resistant Genes -Glycin-Rich Proteins, Wall-Associated Receptor Kinase (Wak) -Proline Oxidase
Down-Regulated	Repressors Of BA	-Regulation Of	-Transfactors (Such As



II.E. GIBBERELIC ACID RESPONSIVE GENES, GENE COMPONENTS AND  
PRODUCTS

Plant hormones are naturally occurring substances, effective in very small amounts, which  
5 act as signals to stimulate or inhibit growth or regulate developmental processes in plants.  
Gibberellic acid (GA) is a hormone in vascular plants that is synthesized in proplastids (giving  
rise to chloroplasts or leucoplasts) and vascular tissues. The major physiological responses  
affected by GA are seed germination, stem elongation, flower induction, anther development and  
seed and pericarp growth. GA is similar to auxins, cytokinins and gibberellins, in that they are  
10 principally growth promoters.

Changes in GA concentration in the surrounding environment or in contact with a plant  
result in modulation of many genes and gene products. Examples of such GA responsive genes  
and gene products are shown in the Reference and Sequence Tables. These genes and/or  
products are responsible for effects on traits such as plant vigor and biomass and seed yield.  
15 They were discovered and characterized from a much larger set of genes by experiments  
designed to find genes whose mRNA products changed in concentration in response to  
application of nitrogen to plants.

While GA responsive polynucleotides and gene products can act alone, combinations of  
these polynucleotides also affect growth and development. Useful combinations include  
20 different GA responsive polynucleotides and/or gene products that have similar transcription  
profiles or similar biological activities, and members of the same or similar biochemical  
pathways. Whole pathways and/or segments of pathways are controlled by transcription factors  
and proteins that affect the activity of signal transduction pathways. Therefore, manipulation of  
such protein levels is especially useful for altering phenotypes and biochemical activities of  
25 plants. In addition, the combination of a GA responsive polynucleotide and/or gene product with  
another environmentally responsive polynucleotide is also useful because of the interactions that  
exist between hormone regulated pathways, stress pathways, nutritional pathways and  
development. Here, in addition to polynucleotides having similar transcription profiles and/or  
biological activities, useful combinations include polynucleotides that may have different  
30 transcription profiles but which participate in common overlapping pathways. The MA\_diff  
and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript

levels of the experiment (see EXPT ID: 108562, 108563, 108519, 108520, 108521, 108484, 108485, 108486). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

GA genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### GA Genes Identified By Cluster Analyses Of Differential Expression

##### GA Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of GA genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108562, 108563, 108519, 108520, 108521, 108484, 108485, 108486 of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

##### GA Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of GA genes. A group in the MA\_clust is considered a GA pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

##### GA Genes Identified By Amino Acid Sequence Similarity

GA genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis GA genes. Groups of GA

genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a GA pathway or network is a group of proteins that also exhibits GA functions/utilities.

5           Such GA responsive genes and gene products can function to either increase or dampen the above phenotypes or activities either in response to changes in GA concentration or in the absence of GA fluctuations. Further, promoters of GA responsive genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by GA or any of the following phenotypes or biological activities below.

10                           II.E.1. USE OF GA RESPONSIVE GENES TO MODULATE PHENOTYPES:

GA responsive genes and gene products are useful to or modulate one or more of the following phenotypes:

- 15
- Growth
  - Promotes Root Growth
  - Promotes Cell Division
  - Promotes Stem Elongation
  - Secondary (Woody) Growth
  - Promotes Growth In Leaves
  - 20 • Biomass
  - Increase In Stem And Leaf Mass
  - Increase In Xylem Fiber Length And Biomass Production
  - Development
  - Cell Growth
  - 25 • Fruit Development
  - Seed Development
  - Dormancy, Breaks Dormancy In Seeds And Buds
  - Promotes Trichome Formation
  - Decrease Senescence
  - 30 • Regulation Of Fertility
  - Stress Responses

- Flowering Time

Further, any desired sequence can be transcribed in similar temporal, tissue, or enviromentally specific patterns as the GA responsive genes when the desired sequence is operably linked to a promoter of a GA responsive gene.

To regulate any of the phenotype(s) above, activities of one or more of the GA response genes or gene products can be modulated and tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266) and visually inspected for the desired phenotype or metabolically and/or functionally assayed according to Hedden and Proebsting (1999, Plant Physiol. 119:365-370), Hedden and Phillips (1999, Current Opinion in Plant Biotech. 11:130-137), Perazza et al (1998, Plant Physiol. 117:375-383), Kende and Zeevart (1997, Plant Cell 9:1197-1210) and van der Knaap et al. (2000, Plant Physiol. 122:695-704).

#### II.E.2. USE OF GA-RESPONSIVE GENES TO MODULATE BIOCHEMICAL ACTIVITIES:

The activities of one or more of the GA responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Cell Growth and Differentiation	Biosynthesis of Gas	Hedden and Proebsting (1999, Plant Physiol. 119:365-370)
	Cell wall loosening and cell expansion	Cosgrove (1993, New Phytol. 124:1-23)

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	GA deactivation Major growth promoting metabolic pathways	Hedden and Proebsting (1999, Plant Physiol. 119:365-370)
Perception and Signal Transduction	Receptors	Koornneef and van der Veen (1980, TAG 58:257- 263)
	Synthesis of transcriptional regulators Calcium and Calmodulin	Bethke and Jones (1998, Curr. Opin. Plant Biol. 1:440-446)

Other biological activities that can be modulated by the GA responsive genes and gene products are listed in the Reference Tables. Assays for detecting such biological activities are described in the Protein Domain table.

GA responsive genes are characteristically differentially transcribed in response to fluctuating GA levels or concentrations, whether internal or external to an organism or cell. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s) report(s) the changes in transcript levels of various GA responsive genes in entire seedlings at 1 and 6 hours after a plant was sprayed with a Hoagland's solution enriched with GA as compared to seedlings sprayed with Hoagland's solution only.

The data from this time course can be used to identify a number of types of GA responsive genes and gene products, including "early responders," and "delayed responders." Profiles of some GA responsive genes are shown in the Table below with examples of associated biological activities.

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY



TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
Up regulated transcripts (level at 1 hr $\approx$ 6 hr) (level at 1 hr > 6 hr)	Early responders to GA  Genes induced by GA	GA perception GA transport Modulation of GA response transduction pathways Specific gene transcription initiation Growth stimulating pathway induction	Transcription factors Transporters Change in cell membrane structure Kinases and phosphatases  Transcription activators Change in chromatin structure and/or localized DNA topology Cell wall proteins Metabolic Enzymes
Up regulated transcripts (level at 1 hr < 6 hr)	Maintenance of GA response  "Delayed" responders	Maintenance of response to GA Induction of GA metabolic pathways	Transcription factors Specific factors (initiation and elongation) for protein synthesis Maintenance of mRNA stability Metabolic enzymes
Down-regulated transcripts (level at 1 hr $\approx$ 6 hr) (level at 6 hr > 1 hr)	Early repressor responders to GA  Genes repressed by GA	Negative regulation of GA pathways released Reduced activity of repressed pathways	Transcription factors Calmodulin Change in protein structure by phosphorylation (kinases) or dephosphorylation

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
	Genes whose activities are diminished or mRNAs are unstable in the presence of GA		(phosphatases) Change in chromatin structure and/or DNA topology
Down-regulated transcripts (level at 1 hr > 6 hr)	Delayed responders  Genes repressed by GA  Genes whose activities are diminished or mRNAs are unstable in the presence of GA	Maintenance or GA repressed pathways	Transcription factors Kinases and phosphatases Stability factors for protein translation Metabolic enzymes

#### Use of Promoters of GA responsive Genes

Promoters of GA responsive genes are useful for transcription of any desired polynucleotide or plant or non-plant origin. Further, any desired sequence can be transcribed in a similar temporal, tissue, or environmentally specific patterns as the GA responsive genes where the desired sequence is operably linked to a promoter of a GA responsive gene. The protein product of such a polynucleotide is usually synthesized in the same cells, in response to the same stimuli as the protein product of the gene from which the promoter was derived. Such promoters are also useful to produce antisense mRNAs to down-regulate the product of proteins, or to produce sense mRNAs to down-regulate mRNAs via sense suppression.

III. METABOLISM AFFECTING GENES, GENE COMPONENTS AND PRODUCTS

III.A. NITROGEN RESPONSIVE GENES, GENE COMPONENTS AND PRODUCTS

Nitrogen is often the rate-limiting element in plant growth, and all field crops have a  
5 fundamental dependence on exogenous nitrogen sources. Nitrogenous fertilizer which is usually  
supplied as ammonium nitrate, potassium nitrate, or urea, typically accounts for 40% of the costs  
associated with crops, such as corn and wheat in intensive agriculture. Increased efficiency of  
nitrogen use by plants should enable the production of higher yields with existing fertilizer inputs  
and/or enable existing yields of crops to be obtained with lower fertilizer input, or better yields on  
10 soils of poorer quality. Also, higher amounts of proteins in the crops could also be produced more  
cost-effectively.

Changes in nitrogen concentration in the surrounding environment or in contact with a plant  
results in modulation of the activities of many genes and hence levels of gene products. Examples  
of such "nitrogen responsive" genes and gene products with these properties are shown in the  
15 Reference, Sequence, Protein Group, Protein Group Matrix tables, MA\_diff and/or AFLP\_diff  
MA\_diff and/or AFLP\_diff and/or AFLP\_diff, MA\_clust, Knock-in and Knock-out tables. These  
genes and/or products are responsible for effects on traits such as plant vigor and seed yield. They  
were discovered and characterized from a much larger set by experiments designed to find genes  
whose mRNA products changed in response to changing levels of available nitrogen to plants.

20 Manipulation of one or more "nitrogen responsive" gene activities are useful to modulate  
the biological activities and/or phenotypes listed below. "Nitrogen responsive" genes and gene  
products can act alone or in combination. Useful combinations include nitrogen responsive  
genes and/or gene products with similar transcription profiles, similar biological activities, or  
members of the same or functionally related biochemical pathways. Whole pathways or  
25 segments of pathways are controlled by transcription factor proteins and proteins controlling the  
activity of signal transduction pathways. Therefore, manipulation of the levels of such proteins is  
especially useful for altering phenotypes and biochemical activities of plants. The MA\_diff  
and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript  
levels of the experiment (see EXPT ID: 108592, 108593, 108588, 108589, 108590, 108591,  
30 108532, 108548, 108549, 108550, 108551, 108454, 108455, 108487, 108488, 108489, and  
Nitrogen (relating to SMD 3787, SMD 3789)). For transcripts that had higher levels in the

samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Nitrogen genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### Nitrogen Genes Identified By Cluster Analyses Of Differential Expression

##### Nitrogen Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Nitrogen genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108592, 108593, 108588, 108589, 108590, 108591, 108532, 108548, 108549, 108550, 108551, 108454, 108455, 108487, 108488, 108489, and Nitrogen (relating to SMD 3787, SMD 3789) of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

##### Nitrogen Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Nitrogen genes. A group in the MA\_clust is considered a Nitrogen pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

##### Nitrogen Genes Identified By Amino Acid Sequence Similarity

Nitrogen genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Nitrogen genes. Groups of Nitrogen genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Nitrogen pathway or network is a group of proteins that also exhibits Nitrogen functions/utilities.

Such "nitrogen responsive" genes and gene products can function either to either increase or dampen the phenotypes and activities below, either in response to changes in nitrogen concentration or in the absence of nitrogen fluctuations.

Further, promoters of nitrogen responsive genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by nitrogen or any of the following phenotypes or biological activities below.

#### III.A.1.USE OF NITROGEN-RESPONSIVE GENES TO MODULATE PHENOTYPES

"Nitrogen responsive" genes and gene products can be used to alter or modulate one or more of the following phenotypes:

- Plant Development
- Initiation of the Reproduction Cycle from a Vegetative State
  - Flower Development Time
  - Time to Fruit Maturity; and
- Root Development and Initiation
  - Root Branching
  - Lateral Root, Initiation and/or Development
  - Nodule formation and nitrogen Assimilation from any Nitrogen-Fixing Symbions.
- Growth Rate
- Whole Plant, including Height, Flowering Time, etc.
- Organs
  - Flowers
  - Fruits

- Stems
- Leaves
- Roots
- Lateral Roots
- 5 - Biomass
- Fresh and Dry Weight during any time in plant life, such as maturation;
- Number, Size, and Weight of
  - Flowers;
  - Seeds;
  - 10 - Branches;
  - Leaves;
- Total Plant Nitrogen Content
- Amino Acid/Protein Content of Whole Plant or Parts
- Seed Yield
  - 15 - Number, Size, Weight, Harvest Index
  - Content and Composition, e.g., Amino Acid, Nitrogen, Oil, Protein, and carbohydrate
- Fruit Yield
  - 20 - Number, Size, Weight, Harvest Index
  - Content and Composition, e.g., Amino Acid, Nitrogen, Oil, Protein, carbohydrate, Water

To regulate any of the phenotype(s) above, activities of one or more of the nitrogen responsive genes or gene products can be modulated and the plants can be tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant  
25 utilizing the procedures described herein and the phenotypes can be screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and assayed, for example, in accordance to Zhang (1999) Proc. Natl. Acad. Sci. 96(11): 6529-34; or Zhang and Forde (1998) Science 279(5349):407-9; Scheible, W., Lauerer, M., Schultze, E.-D., Caboche, M., and Sitt, M. (1997). Plant J. 11, 671-691; Chevalier C, Bourgeois E, Just D, Raymond P. Plant J. 1996 Jan;9(1):1-  
30 11.

### III.A.2.USE OF NITROGEN-RESPONSIVE GENES TO MODULATE BIOCHEMICAL ACTIVITIES

The activities of one or more of the nitrogen responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities are documented and can be measured according to the citations included in the Table below:

Process	Biochemical Or Metabolic Activities And/Or Pathways	Citations including assays
Nitrate And Ammonium Uptake and Assimilation	NO <sub>3</sub> <sup>-</sup> Influx And Efflux	Lejay et al. (1999) Plant J. 18(5): 509-519
	Nitrate Channels	Liu et al. (1999) Plant Cell 11: 865-874; and Wang et al.(1998) Proc. Natl. Acad. Sci. USA 95: 15134-15139
	Changes In Membrane-Potential	Meharg et al. (1995) J. Membr. Biol. 145: 49-66; and Wang et al. (1998), supra
Amino Acid Synthesis	Glutamine Synthesis And Then Biosynthesis Of Other Amino Acids	Coruzzi et al. U.S. Pat. No. 5,955,651; and Oliveira et al. (1999) Plant. Phys. 121: 301-309
	Asparagine Synthesis And Then Biosynthesis Of Other Amino Acids	Lam et al. (1998) Plant J. 16(3): 345-353
Coordination Of Carbon And Nitrogen Metabolism	Light-Regulation Of Major Central Carbon And Nitrogen Metabolic Pathways To Coordinate Growth	Lam et al. (1998), supra; Lejay et al. (1999), supra; and Oliveira et al. (1999), supra

Process	Biochemical Or Metabolic Activities And/Or Pathways	Citations including assays
	Carbohydrate And Nitrogen Control Of Carbohydrate And Organic Nitrogen Accumulation Pathways	Lam et al. (1998) supra; Lejay et al. (1999) supra; and Oliveira et al. (1999) supra
Nitrogen Loading And Unloading	Nitrogen Transport From Source To Sinks	Walker et al. (1999) 210(1):9-18 Elsheikh et al. (1997) 51(2):137-44.
Nitrogen Storage	Accumulation Of Amino Acids And/Or Storage Proteins In Vacuoles	Johnson et al. (1990) Plant Cell 2(6):525-32.  Herman and Larkins (1999) Plant Cell. 11(4):601-14.
Ammonium Detoxification	Plastid Ammonium Storage/Glutamine Synthesis	Crawford (1995) Plant Cell 7(7):859-68. Zhang and Forde (1998) Science 279: 407-409.
Cell Growth	Division And/Or Elongation	Zhang and Forde (1998) Science 279: 407-409.  Coruzzi et al. U.S. Pat. No. 5,955,651

Other biological activities that can be modulated by the nitrogen responsive genes and their products are listed in the Reference tables. Assays for detecting such biological activities are described in the Domain section above.

- 5 Nitrogen responsive genes are characteristically differentially transcribed in response to fluctuating nitrogen levels or concentrations, whether internal or external to an organism or cell. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table reports the changes in transcript levels of various nitrogen responsive genes in the aerial parts of a seedling at 2, 6, 9 and 12 hours after a plant was sprayed with a solution enriched with ammonium nitrate
- 10 as compared to seedlings sprayed with water. The MA\_diff and/or AFLP\_diff MA\_diff and/or



AFLP\_diff and/or AFLP\_diff reports the changes in transcript levels of various nitrogen responsive genes in roots at 12 and 24 hours that were cut from seedlings transferred from a high to low potassium nitrate environment compared to control seedlings transferred to a high potassium nitrate environment.

- 5           The data from this time course reveal a number of types of nitrogen responsive genes and gene products, including "early responders," and "delayed nitrogen responders". Profiles of the individual categories of nitrogen responsive genes are shown in the Tables below together with examples of the kinds of associated biological activities that are modulated when the activities of one or more such genes vary in plants.

Low to High Ammonium Nitrate Experiment

Gene Expression Levels	Functional Category Of Gene	Physiological Consequences	Examples Of Gene Products
Upregulated Transcripts (Level At 2h $\cong$ 6, 9 Or 12h) Or (Level At 2h > 6, 9 Or 12h)	Early Responders To Nitrogen	<ul style="list-style-type: none"> <li>- Perception Of Nitrogen</li> <li>- Induced Nitrogen Uptake Into Cells</li> <li>- Induction Of Nitrogen Response Transduction Pathways</li> <li>- Initiation Of Specific Gene Transcription</li> </ul>	<ul style="list-style-type: none"> <li>- Transcription Factors</li> <li>- Transporters</li> <li>- Inhibitors Of Nitrogen Fixation</li> <li>- Components Of Pathways Released From Repression</li> <li>- Transaminases</li> <li>- Amino Acid Biosynthetic Enzymes</li> </ul>
Upregulated Transcripts (Level At 2h < 6, 9, Or 12h)	Delayed Nitrogen Responders	<ul style="list-style-type: none"> <li>- Maintenance Of High Nitrogen Metabolism And Growth</li> </ul>	<ul style="list-style-type: none"> <li>- Nitrogen Metabolic Pathway Enzymes</li> <li>- Transaminases</li> <li>- Amino Acid Biosynthetic Enzymes</li> <li>- Factors Induced In Coordination And Control Of Central Carbon And Nitrogen Metabolism</li> <li>- Cell Wall And Cell Growth- Promoting Pathway Enzymes</li> <li>- Storage Proteins</li> </ul>

Gene Expression Levels	Functional Category Of Gene	Physiological Consequences	Examples Of Gene Products
Down Regulated Transcripts (Level At 2h $\cong$ 6, 9 Or 12h) Or (Level At 6, 9 Or 12h > 2h)	<ul style="list-style-type: none"> <li>- Early Responder Repressors Of Nitrogen Utilization Pathways</li> <li>- Genes With Discontinued Expression Or UnsTable Mrna Following Nitrogen Uptake</li> </ul>	<ul style="list-style-type: none"> <li>- Negative Regulation Of Nitrogen Utilization Pathways Released</li> <li>- Pathways Of C And N Metabolism Required At Lower Levels Decline In Presence Of High Nitrogen</li> </ul>	<ul style="list-style-type: none"> <li>- Transcription Factors</li> <li>- Kinases And Phosphatases</li> <li>- Cytoskeletal Proteins Modulating Cell Structure</li> <li>- Chromatin Structure</li> <li>- Regulatory Proteins</li> <li>- Metabolic Enzymes</li> <li>-Transporters</li> <li>- Proteins And Rna Turnover Systems</li> </ul>
Level At 2 Hours > 6,9 Or 12 Hours	<ul style="list-style-type: none"> <li>- Delayed Response Repressors Of Nitrogen Utilization Pathways</li> <li>- Genes With Discontinued Expression Or UnsTable Mrna Following Nitrogen Uptake</li> </ul>	<ul style="list-style-type: none"> <li>- Negative Regulation Of Nitrogen Utilization Pathways Released</li> <li>- Pathways Of C And N Metabolism Required At Lower Levels Decline In Presence Of High Nitrogen</li> </ul>	<ul style="list-style-type: none"> <li>- Transcription Factors</li> <li>- Kinases And Phosphatases</li> <li>- Cytoskeletal Proteins Modulating Cell Structure</li> <li>- Chromatin Structure</li> <li>- Regulatory Proteins</li> <li>- Metabolic Enzymes</li> <li>- Transporters</li> <li>- Protein And Rna Turnover Systems</li> </ul>

### High to Low Potassium Nitrate Experiments

Gene Expression Levels	Functional Category Of Gene	Type Of Biological Activity	Examples Of Biochemical Activities Of Gene Products
Upregulated Transcripts (Level At 12h ~ 24h) (Level At 12h>24h)	Early Responders To Low Nitrate	<ul style="list-style-type: none"> <li>- Perception Of Low Nitrate</li> <li>- Nitrogen Uptake Into Cells</li> <li>- Low Nitrogen Signal Transduction Response Pathways</li> <li>- Initiation Of Specific Gene Transcription</li> <li>- Initiation Of Nitrogen Fixation</li> </ul>	<p>Transcription Factors – Controlling Transcription</p> <p>Transporters – Facilitating Transport</p> <p>Cell Wall/Membrane structure Determining proteins</p> <p style="text-align: right;">Kinases And Phosphatases-regulating Signal Transduction Pathways</p> <p style="text-align: right;">Cytoskeletal Proteins- Modulating Cell Structure</p> <p style="text-align: right;">Chromatin Structure And/Or Dna Topology Proteins</p> <p>Protein-Protein Interaction participants</p> <p>Metabolic Enzymes-nitrogen Turnover Enzymes and Pathway Components</p>

Gene Expression Levels	Functional Category Of Gene	Type Of Biological Activity	Examples Of Biochemical Activities Of Gene Products
Upregulated Transcripts (Level 12h<24h)	Delayed Low Nitrate Responders	- Maintenance Of Low Nitrogen Response Pathways (See the Table Above)	<p>Transcription Factors – Controlling Transcription</p> <p>Transporters – Facilitating Transport</p> <p>Cell Wall/Membrane structure Determining proteins</p> <p style="text-align: center;">Kinases And Phosphatases-regulating Signal transduction Pathways</p> <p style="text-align: center;">Cytoskeletal Proteins- Modulating Cell Structure</p> <p style="text-align: center;">Chromatin Structure And/Or Dna Topology Proteins</p> <p>Protein-Protein Interaction participants</p> <p>Metabolic Enzymes-nitrogen Turnover Enzymes and Pathway Components</p>

Gene Expression Levels	Functional Category Of Gene	Type Of Biological Activity	Examples Of Biochemical Activities Of Gene Products
Down-Regulated Transcripts (Level At 12h~24h) (Level At 12h>24h)	- Early Repressor Responders To Low Nitrate  - Genes Whose Expression Is Discontinued Or Mrna Is Unstable In Presence Of Low Nitrate	-Negative Regulation Of Low Nitrogen-Mediated Pathways And/Or Responses Released  - Pathways In C And N Metabolism Required At Lower Levels Decline In The Presence Of Low Nitrate	- Transcription Factors  Cell Wall/Membrane Structure Determining Proteins  Factors For Promoting Protein Translation  Kinases And Phosphatases  Cytoskeletal Proteins- modulating Cell Structure Protein And Rna Turnover systems

Gene Expression Levels	Functional Category Of Gene	Type Of Biological Activity	Examples Of Biochemical Activities Of Gene Products
Down-Regulated Transcripts (Level At 12h<24h)	- Delayed Repressor Responders To Low Nitrate  - Genes Whose Expression Is Discontinued Or mRNA Is Unstable In Presence Of Low Nitrate	Negative Regulation Of Low Nitrogen-Mediated Pathways And/Or Responses Released Pathways In C And N Metabolism Required At Lower Levels Decline In The Presence Of Low Nitrate	Transcription Factors Cell Wall/Membrane Structure Determining Proteins Factors For Promoting Protein Translation Kinases And Phosphatases Cytoskeletal Proteins- modulating Cell Structure Protein And Rna Turnover systems Chromatin Structure and/Or Dna Topology proteins

Further, any desired sequence can be transcribed in similar temporal, tissue, or environmentally specific patterns as the nitrogen responsive genes when the desired sequence is operably linked to a promoter of a nitrogen responsive gene.

III.B. CIRCADIAN RHYTHM RESPONSIVE GENES, GENE COMPONENTS AND PRODUCTS

Often growth and yield are limited by the ability of a plant to tolerate stress conditions, including water loss. To combat such conditions, plant cells deploy a battery of responses that are controlled by an internal circadian clock, including the timed movement of cotyledons and leaves, timed movements in guard cells in stomata, and timed biochemical activities involved with sugar and nitrogen metabolism. These responses depend on the functioning of an internal circadian clock, that becomes entrained to the ambient light/dark cycle, and a series of downstream signaling events leading to transcription independent and transcription dependent stress responses.

A functioning circadian clock can anticipate dark/light transitions and prepare the physiology and biochemistry of a plant accordingly. For example, expression of a chlorophyll a/b binding protein (CAB) is elevated before daybreak, so that photosynthesis can operate maximally as soon as there is light to drive it. Similar considerations apply to light/dark transitions and to many areas of plant physiology such as sugar metabolism, nitrogen metabolism, water uptake and water loss, flowering and flower opening, epinasty, germination, perception of season, and senescence.

Manipulation of one or more clock gene activities are useful to modulate the biological processes and/or phenotypes listed below. Clock responsive genes and gene products can act alone or in combination. Useful combinations include clock responsive genes and/or gene products with similar transcription profiles, similar biological activities, or members of the same or functionally related biochemical pathways. Whole pathways or segments of pathways are controlled by transcription factor proteins and proteins controlling the activity of signal transduction pathways. Therefore, manipulation of such protein levels is especially useful for altering phenotypes and biochemical activities of plants. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: Circadian (relating to SMD 2344, SMD 2359, SMD 2361, SMD 2362, SMD 2363, SMD 2364, SMD 2365, SMD 2366, SMD 2367, SMD 2368, SMD 3242)). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is



shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Circadian genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### Circadian Genes Identified By Cluster Analyses Of Differential Expression

##### Circadian Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Circadian genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID Circadian (relating to SMD 2344, SMD 2359, SMD 2361, SMD 2362, SMD 2363, SMD 2364, SMD 2365, SMD 2366, SMD 2367, SMD 2368, SMD 3242) of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

##### Circadian Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Circadian genes. A group in the MA\_clust is considered a Circadian pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

#### Circadian Genes Identified By Amino Acid Sequence Similarity

Circadian genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Circadian genes.

Groups of Circadian genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Circadian pathway or network is a group of proteins that also exhibits Circadian functions/utilities.

Such clock responsive genes and gene products can function to either increase or dampen the above phenotypes or activities either in response to changes in daylength or in response to changes in light quality. Further, promoters of circadian (clock) responsive genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by circadian or any of the following phenotypes or biological activities below. Further, any desired sequence can be transcribed in similar temporal, tissue, or environmentally specific patterns as the circadian (clock) responsive genes when the desired sequence is operably linked to a promoter of a circadian (clock) responsive gene.

The expression of many genes is modulated by the clock. Microarray technology allows monitoring of gene expression levels for thousands of genes in a single experiment. This is achieved by hybridizing labeled fluorescent cDNA pools to glass slides that contain spots of DNA (Schena et al. (1995) Science 270: 467-70). The US Arabidopsis Functional Genomics Consortium (AFGC) has recently made public the results from such microarray experiments conducted with AFGC chips containing some 10,000 non-redundant ESTs, selected from about 37,000 randomly sequenced ESTs generated from mRNA of different tissues and developmental stages.

The sequences of the ESTs showing at least two-fold increases or decreases in response to the circadian rhythm clock at various times through the 24 hour cycle relative to the controls were identified, compared to the Ceres full length cDNA and genomic sequence databanks, and equivalent Ceres clones identified. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table reports the results of this analysis, indicating those Ceres clones which are up or down regulated over controls, thereby indicating the Ceres clones which represent clock responsive genes.

### III.B.1. USE OF CIRCADIAN RHYTHM (CLOCK) RESPONSIVE GENES TO MODULATE PHENOTYPES

Clock responsive genes and gene products are useful to or modulate one or more of the following phenotypes:

- Timing Phenotypes
- Dormancy
- Germination
- Cotyledon opening
- First leaves
- Juvenile to adult transition
- Bolting
- Flowering
- Pollination
- Fertilization
- Seed development
- Seed set
- Fruit Drop
- Senescence
- Epinasty
- Biomass
- Fresh and Dry Weight during any time in plant life, such as maturation
- Number of Flowers, Seeds, Branches, and/or Leaves
- Seed Yield, including Number, Size, Weight, and/or Harvest Index
- Fruit Yield, including Number, Size, Weight, and/or Harvest Index
- Plant Development
- Time to Fruit Maturity
- Cell Wall Strengthening and Reinforcement
- Stress Tolerance
- Drought tolerance
- Flooding tolerance
- UV tolerance

To regulate any of the phenotype(s) above, activities of one or more of the clock responsive genes or gene products can be modulated and the plants can be tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be screened for variants as in

5 Anderson et al. (1997) Plant Cell 9: 1727-1743; Heintzen et al. (1997) Proc. Natl. Acad. Sci. USA 94: 8515-20; Schaffer et al. (1998) Cell 93:1219-1229; Somers et al. (1998) Development 125: 485-494; Somers et al. (1998) Science 282: 1488-1490; Wang and Tobin (1998) Cell 93: 1207-1217; Zhong et al. (1998) Plant Cell 10: 2005-2017; Sugano et al. (1998) Proc. Natl. Acad. Sci. USA 95: 11020-11025; Dowson-Day and Millar (1999) Plant J 17: 63-71; Green and Tobin

10 (1999) Proc. Natl. Acad. Sci. USA 96: 4176-419; Staiger and Apel (1999) Mol. Gen. Genet. 261: 811-819; Strayer and Kay (1999) Curr. Opin. Plant Biol. 2:114-120; Strayer et. al. (2000) Science 289:768-771; Kreps et al. (2000) J Biol Rhythms (2000) 15:208-217; Nelson et al. (2000) Cell 101:331-340; Somers et al. (2000) Cell 101:319-329.

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III.B.2. USE OF ACTIVE CLOCK RESPONSIVE GENES TO MODULATE  
BIOCHEMICAL ACTIVITIES

The activities of one or more of the clock responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities are documented and can be measured according to the citations above and included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Germination and seedling development	Cold, light and water modulated signal transduction pathways, receptors, kinases, PAS domain	Bognar et al. (1999) Proc. Natl. Acad. Sci. USA 96:14652-14657; Sugano et al (1999) Proc. Natl. Acad. Sci. USA 96:12362-12366; Dowson-Day and Millar (1999) Plant J 17: 63-71; Somers et al. (2000) Cell 101:319-329; Zhong et al. (1998) Plant Cell 10: 2005- 2017
Growth transitions and flowering	Cold and light modulated signal transduction pathways, receptors, kinases, PAS domain	Nelson et al. (2000) Cell 101:331-340; Fowler et al. (1999) EMBO J. 18:4679- 4688
Tuber formation	Cold and light modulated signal transduction pathways	Yanovsky et al. (2000) Plant J. 23: 223-232
<u>METABOLISM</u>		
Lipid metabolism	Membrane lipid synthesis including omega-3 fatty acid desaturase, lipases, lipid transfer proteins	Bradley and Reddy (1997) J. Bacteriol. 179: 4407-4410; Martin, M et al. 1999 Europe J. Biochem 262: 283-290

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Sugar metabolism	Glycosylhydrolases, glycosyltransferases, amylases, sucrose synthase, CAB, Rubisco, light signal transduction	Liu et al. (1996) Plant Physiol. 112:43-51; Millar and Kay (1996) Proc Natl Acad Sci U S A 93:15491-15496; Wang et al. (1997) Plant Cell 9:491-507; Shinohara et al (1999) J. Biol. Chem. 273: 446-452
Nitrogen metabolism	Aminotransferases, arginase, proteases and vegetative storage proteins, aromatic amino acid synthesis	Bradley and Reddy (1997) J. Bacteriol. 179: 4407-4410
Photorespiration	Mitochondrial, chloroplast and peroxisomal photorespiratory enzymes, serine hydroxymethyl transferases, catalase	Zhong and McClung (1996) Mol. Gen. Genet. 251:196-203; McClung (1997) Free. Radic. Biol. Med. 23:489-496; McClung et al. (2000) Plant Physiol. 123:381-392
Responses to Environmental Stress	Expression of genes involved in responses to drought, salt, UV	McClung (1997) Free Radic Biol Med 23:489-496; Shi et al. (2000) Proc. Natl. Acad. Sci. USA 97:6896-6901

Other biological activities that can be modulated by the clock responsive genes and their products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table.

Clock responsive genes are characteristically differentially transcribed in response to fluctuations in an entrained oscillator, which is internal to an organism and cell. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s) report(s) the changes in transcript levels of various clock responsive genes in a plant.

- 5 Profiles of clock responsive genes are shown in the table below with examples of which associated biological activities are modulated when the activities of one or more such genes vary in plants.

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
Up regulated transcripts	<p>Responders to circadian rhythm</p> <p>Genes induced by rhythm</p>	<ul style="list-style-type: none"> <li>• Circadian rhythm perception</li> <li>• Metabolisms affected by Circadian rhythm</li> <li>• Synthesis of secondary metabolites and/or proteins</li> <li>• Modulation of clock response transduction pathways</li> <li>• Specific gene transcription initiation</li> </ul>	<ul style="list-style-type: none"> <li>• Metabolic enzymes</li> <li>• Change in cell membrane structure and potential</li> <li>• Kinases and phosphatases</li> <li>• Transcription activators</li> <li>• Change in chromatin structure and/or localized DNA topology</li> <li>• Enzymes in lipid, sugar and nitrogen metabolism</li> <li>• Enzymes in photorespiration and photosynthesis</li> </ul>

Down-regulated transcripts	<p>Responders to circadian rhythm.</p> <p>Repressors of circadian "state" of metabolism</p> <p>Genes repressed by rhythm</p> <p>Genes with discontinued expression or unstable mRNA in presence of zinc</p>	<ul style="list-style-type: none"> <li>• Negative regulation of circadian pathways released</li> <li>• Changes in pathways and processes operating in cells</li> <li>• Changes in metabolism other than circadian pathways</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription factors</li> <li>• Change in protein structure by phosphorylation (kinases) or dephosphorylation (phosphatases)</li> <li>• Change in chromatin structure and/or DNA topology</li> <li>• Stability of factors for protein synthesis and degradation</li> <li>• Metabolic enzymes in light, sugar, lipid and nitrogen metabolism</li> </ul>
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#### Use of Promoters of Clock responsive Genes

Promoters of Clock responsive genes are useful for transcription of any desired polynucleotide of plant or non-plant origin. Further, any desired sequence can be transcribed in a similar temporal, tissue, or environmentally specific patterns as the Clock responsive genes where the desired sequence is operably linked to a promoter of a Clock responsive gene. The protein product of such a polynucleotide is usually synthesized in the same cells, in response to the same stimuli as the protein product of the gene from which the promoter was derived. Such promoters are also useful to produce antisense mRNAs to down-regulate the product of proteins, or to produce sense mRNAs to down-regulate mRNAs via sense suppression.



III.C. BLUE LIGHT (PHOTOTROPISM) RESPONSIVE GENES, GENE  
COMPONENTS AND PRODUCTS

Phototropism is the orientation or growth of a cell, an organism or part of an organism in  
5 relation to a source of light. Plants can sense red (R), far-red (FR) and blue light in their  
environment and respond differently to particular ratios of these. For example, a low R:FR ratio  
enhances cell elongation and favors flowering over leaf production, but blue light regulated  
cryptochromes also appear to be involved in determining hypocotyl growth and flowering time.

Phototropism of *Arabidopsis thaliana* seedlings in response to a blue light source is  
10 initiated by nonphototropic hypocotyl 1 (NPH1), a blue light-activated serine-threonine protein  
kinase, but the downstream signaling events are not entirely known. Blue light treatment leads  
to changes in gene expression. These genes have been identified by comparing the levels of  
mRNAs of individual genes in dark-grown seedlings, compared with in dark grown seedlings  
treated with 1 hour of blue light. Auxin also affects blue light phototropism. The effect of auxin  
15 on gene expression stimulated by blue light has been explored by studying mRNA levels in a  
mutant of *Arabidopsis thaliana* *nph4-2*, grown in the dark and, treated with blue light for 1 hour  
compared with wild type seedlings treated similarly. This mutant is disrupted for auxin-related  
growth and auxin-induced gene transcription. Gene expression was studied using microarray  
technology.

20 Microarray technology allows monitoring of gene expression levels for thousands of  
genes in a single experiment. This is achieved by hybridizing labeled fluorescent cDNA pools to  
glass slides that contain spots of DNA (Schena et al. (1995) Science 270: 467-70). The US  
*Arabidopsis* Functional Genomics Consortium (AFGC) has recently made public the results from  
such microarray experiments conducted with AFGC chips containing some 10,000 non-  
25 redundant ESTs, selected from about 37,000 randomly sequenced ESTs generated from mRNA  
of different tissues and developmental stages.

The sequences of the ESTs showing at least two-fold increases or decreases over the  
controls were identified, compared to the Ceres full-length cDNA and genomic sequence  
databanks, and the equivalent Ceres clones identified. The MA\_diff and/or AFLP\_diff MA\_diff  
30 and/or AFLP\_diff and/or AFLP\_diff table(s) report(s) the results of this analysis, indicating  
those Ceres clones which are up or down regulated over controls, thereby indicating the Ceres

clones which represent blue light responsive genes and of those which are blue light responsive in the absence of nph4 gene activity. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: Phototropism (relating to SMD 4188, SMD 6617, SMD 6619)). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Blue Light genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### Blue Light Genes Identified By Cluster Analyses Of Differential Expression

##### Blue Light Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Blue Light genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID Phototropism (relating to SMD 4188, SMD 6617, SMD 6619) of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

##### Blue Light Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Blue Light genes. A group in the MA\_clust is considered a Blue Light pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

Blue Light Genes Identified By Amino Acid Sequence Similarity

Blue Light genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Blue Light genes.

5 Groups of Blue Light genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Blue Light pathway or network is a group of proteins that also exhibits Blue Light functions/utilities.

10 III.C.1.USE OF BLUE LIGHT RESPONSIVE GENES, GENE COMPONENTS  
AND PRODUCTS TO MODULATE PHENOTYPES

Changes in blue light in a plant's surrounding environment result in modulation of many genes and gene products. Examples of such blue light response genes and gene products are shown in the REFERENCE and SEQUENCE Tables. These genes and/or products are responsible for effects on traits such as plant vigor and seed yield.

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While blue light responsive polynucleotides and gene products can act alone, combinations of these polynucleotides also affect growth and development. Useful combinations include different blue light responsive polynucleotides and/or gene products that have similar transcription profiles or similar biological activities, and members of the same or similar biochemical pathways. Whole pathways or segments of pathways are controlled by transcription factor proteins and proteins controlling the activity of signal transduction pathways. Therefore, manipulation of such protein levels is especially useful for altering phenotypes and biochemical activities of plants. In addition, the combination of a blue light responsive polynucleotides and/or gene product with other environmentally responsive polynucleotide is also useful because

20 of the interactions that exist between hormone regulated pathways, stress and pathogen induced pathways, nutritional pathways and development. Here, in addition to polynucleotides having similar transcription profiles and/or biological activities, useful combinations include polynucleotides that may have different transcription profiles but which participate in common or overlapping pathways.

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III.C.2.USE OF BLUE LIGHT RESPONSIVE GENES, GENE COMPONENTS  
AND PRODUCTS TO MODULATE PHENOTYPES

Blue light responsive genes and gene products can function to either increase or dampen the above phenotypes or activities either in response to changes in blue light response concentration or in the absence of blue light responsive fluctuations. Further, promoters of blue light responsive genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by blue light or any of the following phenotypes or biological activities below. Further, any desired sequence can be transcribed in similar temporal, tissue, or environmentally specific patterns as the blue light responsive genes when the desired sequence is operably linked to a promoter of a blue light responsive gene.

Blue light responsive genes and gene products can be used to alter or modulate one or more of the following phenotypes:

- Growth
  - Roots
    - Elongation
    - gravitropism
  - Stems
    - Elongation
- Development
  - Cell
    - Growth
    - Elongation
  - Flower
    - Flowering time
  - Seedling
    - Elongation
  - Plant Yield
  - Seed and fruit yield

To regulate any of the phenotype(s) above, activities of one or more of the blue light responsive genes or gene products can be modulated and the plants tested by screening for

the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266) and/or screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and visually inspected for the desired phenotype or metabolically and/or functionally assayed according to Liscum and Briggs (1995, Plant Cell 7: 473-85), Vitha et al. (2000, Plant Physiol 122: 453-61), Stowe-Evance et al. (1998, Plant Physiol 118: 1265-75), Baum et al. (1999, PNAS USA 96: 13554-9), Huala et al. (1997) Science 278: 2120-2123), Kanegae et al. (2000, Plant Cell Physiol 41: 415-23), Khanna et al. (1999, Plant Mol Biol 39: 231-42), Sakai et al. (2000, Plant Cell 12: 225-36), Parks et al (1996, Plant Physiol 110: 155-62) and Janoudi et al. (1997, Plant Physiol 113: 975-79).

### III.C.3.USE OF BLUE LIGHT RESPONSIVE GENES, GENE COMPONENTS AND PRODUCTS TO MODULATE BIOCHEMICAL ACTIVITIES

The activities of one or more of the blue light responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Cell Growth and Development	Cell Elongation <ul style="list-style-type: none"> <li>Seedling</li> <li>Stem</li> </ul>	Liscum and Briggs (1995) Plant Cell 7: 473-85
	<ul style="list-style-type: none"> <li>Root</li> </ul>	Vitha et al. (2000) Plant Physiol 122: 453-61
Signalling	UV light Perception	Liscum and Briggs (1996) Plant Physiol 112: 291-96

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	Far-red/Red light Perception	Parks et al. (1996) Plant Physiol 110: 155-62
	Phosphorylation of cellular and nuclear-localized proteins	Liscum and Briggs (1996) Plant Physiol 112: 291-96
	Activation and Synthesis of Transcription Factors	Sakae et al. (2000) Plant Cell 12: 225-36
	Ca <sup>2+</sup> levels	Baum et al. (1999) PNAS USA 96: 13554-9 Pu and Robinson (1998) J Cell Sci 111: 3197-3207
	Auxin Concentration	Estelle (1998) Plant Cell 10: 1775-8 Reed et al. (1998) Plant Physiol 118: 1369-78
	Inter-photoreceptors	Janoudi et al. (1997) Plant Physiol 113: 975-79

Other biological activities that can be modulated by blue light response genes and their products are listed in the REF Tables. Assays for detecting such biological activities are described in the Domain section of the REF Table.

- 5 The specific genes modulated by blue light, in wild type seedlings and in the mutant deficient in transmitting auxin effects are given in the Reference and Sequence Tables . The kinds of genes discovered and some of their associated effects are given in the Table below.

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
Up regulated transcripts	Responders to no blue light in wild type or to blue light in mutant lacking auxin effects	<ul style="list-style-type: none"> <li>• Blue light perception</li> <li>• Metabolism affected by blue light</li> <li>• Synthesis of secondary metabolites and/or proteins</li> <li>• Modulation of blue light transduction pathways</li> <li>• Specific gene transcription initiation</li> </ul>	<ul style="list-style-type: none"> <li>• Transporters</li> <li>• Metabolic enzymes</li> <li>• Change in cell membrane structure and potential</li> <li>• Kinases and phosphatases</li> <li>• Transcription activators</li> <li>• Change in chromatin structure and/or localized DNA topology</li> </ul>
Down-regulated transcripts	<p>Responders to no blue light in wild type or to blue light in mutants lacking auxin effects</p> <p>Genes with discontinued expression or unstable mRNA during response</p>	<ul style="list-style-type: none"> <li>• Blue light perception</li> <li>• Metabolism affected by blue light</li> <li>• Synthesis of secondary metabolites and/or proteins</li> <li>• Modulation of blue light transduction pathways</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription factors</li> <li>• Change in protein structure by phosphorylation (kinases) or dephosphorylation (phosphatases)</li> <li>• Change in chromatin structure and/or DNA topology</li> <li>• Stability factors for protein synthesis and</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
		<ul style="list-style-type: none"> <li>• Specific gene transcription initiation</li> <li>• Changes in pathways and processes operating in cells</li> <li>• Changes in metabolic pathways other than phototropic blue light responsive pathways</li> </ul>	<ul style="list-style-type: none"> <li>• degradation</li> <li>• Metabolic enzymes</li> </ul>

#### Use of Promoters of Blue Light responsive Genes

Promoters of Blue Light responsive genes are useful for transcription of any desired polynucleotide or plant or non-plant origin. Further, any desired sequence can be transcribed in a similar temporal, tissue, or environmentally specific patterns as the Blue Light responsive genes where the desired sequence is operably linked to a promoter of a Blue Light responsive gene. The protein product of such a polynucleotide is usually synthesized in the same cells, in response to the same stimuli as the protein product of the gene from which the promoter was derived. Such promoter are also useful to produce antisense mRNAs to down-regulate the product of proteins, or to produce sense mRNAs to down-regulate mRNAs via sense suppression.



### III.D. RESPONSIVE GENES, GENE COMPONENTS AND PRODUCTS

There has been a recent and significant increase in the level of atmospheric carbon  
5 dioxide. This rise in level is projected to continue over the next 50 years. The effects of the  
increased level of carbon dioxide on vegetation are just now being examined, generally in large  
scale, whole plant (often trees) experiments. Some researchers have initiated physiological  
experiments in attempts to define the biochemical pathways that are either affected by and/or are  
activated to allow the plant to avert damage from the elevated carbon dioxide levels. A genomics  
10 approach to this issue, using a model plant system, allows identification of those pathways  
affected by and/or as having a role in averting damage due to the elevated carbon dioxide levels  
and affecting growth. Higher agronomic yields can be obtained for some crops grown in  
elevated CO<sub>2</sub>.

Microarray technology allows monitoring of gene expression levels for thousands of  
genes in a single experiment. This is achieved by hybridizing labeled fluorescent cDNA pools to  
glass slides that contain spots of DNA (Schena et al. (1995) Science 270: 467-70). The U.S.  
Arabidopsis Functional Genomics Consortium (AFGC) has recently made public the results from  
such microarray experiments conducted with AFGC chips containing about 10,000 non-  
redundant ESTs, selected from about 37,000 randomly sequenced ESTs generated from mRNA  
20 of different tissues and developmental stages.

The sequences of the ESTs showing at least two-fold increases or decreases in plants  
grown in higher CO<sub>2</sub> levels compared with plants grown at more normal CO<sub>2</sub> levels, were  
compared to the Ceres full length cDNA and genomic sequence databanks, and equivalent Ceres  
clones were identified. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or  
25 AFLP\_diff table reports the results of this analysis, indicating those Ceres clones which are up  
or down regulated over controls, thereby indicating the Ceres clones cDNA sequences that  
change in response to CO<sub>2</sub>.

Examples of CO<sub>2</sub> responsive genes and gene products are shown in the Reference,  
Sequence, Protein Group, Protein Group Matrix tables, MA\_diff and/or AFLP\_diff MA\_diff  
30 and/or AFLP\_diff and/or AFLP\_diff and MA\_clust tables. While CO<sub>2</sub> responsive  
polynucleotides and gene products can act alone, combinations of these polynucleotides also

affect growth and development. Useful combinations include different CO<sub>2</sub> responsive polynucleotides and/or gene products that have similar transcription profiles or similar biological activities, and members of the same or similar biochemical pathways. Whole pathways or segments of pathways are controlled by transcription factor proteins and proteins controlling the activity of signal transduction pathways. Therefore, manipulation of such protein levels is especially useful for altering phenotypes and biochemical activities of plants.

Manipulation of one or more CO<sub>2</sub> responsive gene activities are useful to modulate the biological processes and/or phenotypes listed below. CO<sub>2</sub> responsive genes and gene products can act alone or in combination. Useful combinations include genes and/or gene products with similar transcription profiles, similar biological activities, or members of the same or functionally related biochemical pathways. Here, in addition to polynucleotides having similar transcription profiles and/or biological activities, useful combinations include polynucleotides that may have different transcription profiles but which participate in common or overlapping pathways.

CO<sub>2</sub> responsive genes and gene products can function to either increase or dampen the above phenotypes or activities. Further, promoters of CO<sub>2</sub> responsive genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by CO<sub>2</sub> or any of the following phenotypes or biological activities below. Further, any desired sequence can be transcribed in similar temporal, tissue, or environmentally specific patterns as the CO<sub>2</sub> responsive genes when the desired sequence is operably linked to a promoter of a CO<sub>2</sub> responsive gene. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: CO2 (relating to SMD7561, SMD 7562, SMD 7261, SMD 7263, SMD 3710, SMD 4649, SMD 4650)). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

CO<sub>2</sub> genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### CO2 Genes Identified By Cluster Analyses Of Differential Expression

CO2 Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The  
5 MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of CO2 genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID CO2 (relating to SMD7561, SMD 7562, SMD 7261, SMD 7263, SMD 3710, SMD 4649, SMD 4650) of the MA\_diff and/or AFLP\_diff MA\_diff  
10 and/or AFLP\_diff and/or AFLP\_diff table(s).

CO2 Genes Identified By Correlation To Genes That Cause Physiological  
Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of CO2 genes. A group in the MA\_clust is  
15 considered a CO2 pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

CO2 Genes Identified By Amino Acid Sequence Similarity

CO2 genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis CO2 genes. Groups of CO2 genes are identified in the Protein Group table. In this table, any protein group that  
20 comprises a peptide ID that corresponds to a cDNA ID member of a CO2 pathway or network is  
25 a group of proteins that also exhibits CO2 functions/utilities.

III.D.1.USE OF CO2 RESPONSIVE GENES TO MODULATE PHENOTYPES

CO2 responsive genes and gene products are useful to or modulate one or more of  
30 the following phenotypes:

- Catabolism

- Energy Generation, ATP, etc.
- Metabolism
- Carbohydrate Synthesis
- Growth Rate
- 5     - Whole Plant, including Height, Flowering Time, etc.
- Organs
- Flowers
- Fruits
- Stems
- 10    - Leaves
- Roots
- Lateral Roots
- Biomass
- Fresh and Dry Weight during any time in plant life, such as maturation;
- 15    - Number, Size, and Weight of
- Flowers;
- Seeds;
- Branches;
- Leaves;
- 20    - Total Plant Nitrogen Content
- Amino Acid/Protein Content of Whole Plant or Parts
- Seed Yield
  - Number, Size, Weight, Harvest Index
  - Content and Composition, e.g., Amino Acid, Nitrogen, Oil, Protein, and
  - 25        Carbohydrate
- Fruit Yield
- Number, Size, Weight, Harvest Index
- Content and Composition, e.g., Amino Acid, Nitrogen, Oil, Protein, Carbohydrate, Water
- 30    - Photosynthesis
  - Carbon Dioxide Fixation

To improve any of the phenotype(s) above, activities of one or more of the CO<sub>2</sub> responsive genes or gene products can be modulated and the plants tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a  
5 plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266) and/or screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and visually inspected for the desired phenotype or metabolically and/or functionally assayed according to Saito et al. (1994, Plant Physiol. 106: 887-95), Takahashi et al (1997, Proc. Natl. Acad. Sci. USA 94: 11102-07) and Koprivova et al. (2000, Plant Physiol. 122: 737-46).

### III.D.2.USE OF CO<sub>2</sub> RESPONSIVE GENES TO MODULATE BIOCHEMICAL ACTIVITIES

5 The activities of one or more of the CO<sub>2</sub> responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

GENERAL CATEGORY	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Cell Division	Cell Cycle Control Genes	Masle (2000) Plant Physiol. 122: 1399-1415
Starch Biosynthesis	Starch Biosynthesis Enzymes And Pathways	Ludewig et al., (1998) FEBS Lett. 429: 147-151
Photosynthesis	Photosynthetic Enzymes, e.g., Rubisco	Cheng et al., (1998) Plant Physiol 166: 715-723
Respiration	Energy Metabolism Pathways	Musgrave et al., (1986) Proc. Natl. Acad. Sci. USA 83: 8157-8161
CO <sub>2</sub> Uptake	Guard Cell Stomata Control Systems	Allen et al., Plant Cell (1999) 11(9): 1785-1798 Ichida et al., Plant Cell (1997) 9(10): 1843-1857 Hedrich et al., EMBO J (1993) 12(3): 897-901
Coordination Of Carbon And Nitrogen Metabolism	Light-Regulation Of Major Central Carbon And Nitrogen Metabolic Pathways To Coordinate Growth	Lam et al. (1998) Plant J. 16(3): 345-353 Lejay et al. (1999) Plant J. 18(5): 509-519; and Oliveira et al. (1999) Plant.

GENERAL CATEGORY	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
		Phys. 121: 301-309
	Carbohydrate And Nitrogen Control Of Carbohydrate And Organic Nitrogen Accumulation Pathways	Lam et al. (1998) supra; Lejay et al. (1999) supra; and Oliveira et al. (1999) supra

Other biological activities that can be modulated by the CO<sub>2</sub> responsive genes and gene products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table.

CO<sub>2</sub> responsive genes are characteristically differentially transcribed in response to fluctuating CO<sub>2</sub> levels or concentrations, whether internal or external to an organism or cell. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables report the changes in transcript levels of various CO<sub>2</sub> responsive genes that are differentially expressed in response to high CO<sub>2</sub> levels.

Profiles of these different CO<sub>2</sub> responsive genes are shown in the Table below with examples of associated biological activities.

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
Up Regulated Transcripts	Responders To Higher Levels Of CO <sub>2</sub>	<ul style="list-style-type: none"> <li>Changes In Generation Of ATP</li> <li>Changes In Catabolism And Anabolism Enzymes and Pathways</li> </ul>	<ul style="list-style-type: none"> <li>Transporters</li> <li>Catabolic And Anabolic Enzymes</li> <li>Change In Cell Membrane Structure</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
	Genes Induced By CO <sub>2</sub>	<ul style="list-style-type: none"> <li>• Activation Of Krebs Cycle</li> <li>• Specific Gene Transcription Initiation</li> <li>• Changes In Carbohydrate Synthesis</li> <li>• Changes In Chloroplast Structure</li> <li>• Changes In Photosynthesis</li> <li>• Changes In Respiration</li> </ul>	<p>And Potential</p> <ul style="list-style-type: none"> <li>• Kinases And Phosphatases</li> <li>• Transcription Activators And Repressors</li> <li>• Change In Chromatin Structure And/Or Localized DNA Topology</li> <li>• Redox Control</li> </ul>
Down-Regulated Transcripts	<p>Responders To Higher Levels Of CO<sub>2</sub></p> <p>Genes Repressed By CO<sub>2</sub></p>	<ul style="list-style-type: none"> <li>• Changes In Pathways And Processes Operating In Cells</li> <li>• Changes In Catabolism and Anabolism</li> <li>• Changes in Chloroplast Structure</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> <li>• Change In Protein Structure By Phosphorylation (Kinases) Or Dephosphorylation (Phosphatases)</li> <li>• Change In Chromatin Structure And/Or DNA Topology</li> <li>• Stability Of Factors For Protein Synthesis And Degradation</li> </ul>

104230 523560



TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
			<ul style="list-style-type: none"> <li>Metabolic Enzymes</li> </ul>

#### Use of Promoters of CO<sub>2</sub> responsive Genes

Promoters of CO<sub>2</sub> responsive genes are useful for transcription of any desired polynucleotide or plant or non-plant origin. Further, any desired sequence can be transcribed in a similar temporal, tissue, or environmentally specific patterns as the CO<sub>2</sub> responsive genes where the desired sequence is operably linked to a promoter of a CO<sub>2</sub> responsive gene. The protein product of such a polynucleotide is usually synthesized in the same cells, in response to the same stimuli as the protein product of the gene from which the promoter was derived. Such promoter are also useful to produce antisense mRNAs to down-regulate the product of proteins, or to produce sense mRNAs to down-regulate mRNAs via sense suppression.

III.E. MITOCHONDRIA ELECTRON TRANSPORT (RESPIRATION) GENES,  
GENE COMPONENTS AND PRODUCTS

One means to alter flux through metabolic pathways is to alter the levels of proteins in the pathways. Plant mitochondria contain many proteins involved in various metabolic processes, including the TCA cycle, respiration, and photorespiration and particularly the electron transport chain (mtETC). Most mtETC complexes consist of nuclearly-encoded mitochondrial proteins (NEMPs) and mitochondrially-encoded mitochondrial proteins (MEMPs). NEMPs are produced in coordination with MEMPs of the same complex and pathway and with other proteins in multi- organelle pathways. Enzymes involved in photorespiration, for example, are located in chloroplasts, mitochondria, and peroxisomes and many of the proteins are nuclearly-encoded. Manipulation of the coordination of protein levels within and between organelles can have critical and global consequences to the growth and yield of a plant. Genes which are manipulated by interfering with the mtETC have been characterized using microarray technology.

Microarray technology allows monitoring of gene expression levels for thousands of genes in a single experiment. This is achieved by hybridizing labeled fluorescent cDNA pools to glass slides that contain spots of DNA (Schena et al. (1995) Science 270: 467-70). The US Arabidopsis Functional Genomics Consortium (AFGC) has recently made public the results from such microarray experiments conducted with AFGC chips containing about 10,000 non-redundant ESTs, selected from about 37,000 randomly sequenced ESTs generated from mRNA of different tissues and developmental stages.

The sequences of the ESTs showing at least two-fold increases or decreases in the presence of the ETC inhibitor, 10 mM antimycin A compared with the control lacking antimycin A. were identified, compared to the Ceres full length cDNA and genomic sequence databanks, and equivalent Ceres clones identified. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table reports the results of this analysis, indicating those Ceres clones which are up or down regulated over controls, thereby indicating the Ceres clones that represent respiration responsive genes.

Examples of genes and gene products that are responsive to antimycin A block of respiration are shown in the Reference, Sequence, Protein Group, Protein Group Matrix,

MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff and MA\_clust tables. While respiration responsive polynucleotides and gene products can act alone, combinations of these polynucleotides also affect growth and development. Useful combinations include different respiration responsive polynucleotides and/or gene products that have similar transcription profiles or similar biological activities, and members of the same or similar biochemical pathways. Here, in addition to polynucleotides having similar transcription profiles and/or biological activities, useful combinations include polynucleotides that may have different transcription profiles but which participate in common or overlapping pathways. Whole pathways or segments of pathways are controlled by transcription factor proteins and proteins controlling the activity of signal transduction pathways. Therefore, manipulation of such protein levels is especially useful for altering phenotypes and biochemical activities of plants. Manipulation of one or more respiration responsive gene activities are useful to modulate the biological processes and/or phenotypes listed below.

Such respiration responsive genes and gene products can function to either increase or dampen the phenotypes or activities below. Further, promoters of respiration responsive genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by respiration or any of the following phenotypes or biological activities below. Further, any desired sequence can be transcribed in similar temporal, tissue, or environmentally specific patterns as the respiration responsive genes when the desired sequence is operably linked to a promoter of a respiration responsive gene. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: Mitochondria-Electron Transport (relating to SMD 8061, SMD 8063)). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Mitochondria-Electron Transport genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

Mitochondria-Electron Transport Genes Identified By Cluster Analyses Of Differential Expression

Mitochondria-Electron Transport Genes Identified By Correlation To Genes That  
Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the  
5 microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Mitochondria-Electron Transport genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID Mitochondria-Electron  
10 Transport (relating to SMD 8061, SMD 8063) of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

Mitochondria-Electron Transport Genes Identified By Correlation To Genes That  
Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Mitochondria-Electron Transport genes. A group in the MA\_clust is considered a Mitochondria-Electron Transport pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

Mitochondria-Electron Transport Genes Identified By Amino Acid Sequence Similarity

Mitochondria-Electron Transport genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Mitochondria-Electron Transport genes. Groups of Mitochondria-Electron Transport genes are  
25 identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Mitochondria-Electron Transport pathway or network is a group of proteins that also exhibits Mitochondria-Electron Transport functions/utilities.

III.E.1. USE OF RESPIRATION RESPONSIVE GENES TO MODULATE  
PHENOTYPES

Respiration responsive genes and gene products are useful to or modulate one or more of the following phenotypes:

- Catabolism
- Energy Generation, ATP, etc.
- - Growth Rate
- Whole Plant, including Height, Flowering Time, etc.
- Organs
- Flowers
- Fruits
- Stems
- Leaves
- Roots
- Lateral Roots
- Biomass
- Fresh and Dry Weight during any time in plant life, such as maturation;
- Number, Size, and Weight of
- Flowers;
- Seeds;
- Branches;
- Leaves;
- Total Plant Nitrogen Content
- Amino Acid/Protein Content of Whole Plant or Parts
- Seed Yield
  - Number, Size, Weight, Harvest Index
  - Content and Composition, e.g., Amino Acid, Nitrogen, Oil, Protein, and Carbohydrate
- Fruit Yield
- Number, Size, Weight, Harvest Index

- Content and Composition, e.g., Amino Acid, Nitrogen, Oil, Protein, Carbohydrate, Water
- Photosynthesis
  - Carbon dioxide fixation

To improve any of the phenotype(s) above, activities of one or more of the respiration responsive genes or gene products can be modulated and the plants tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266) and/or screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and visually inspected for the desired phenotype or metabolically and/or functionally assayed according to Saito et al. (1994, Plant Physiol. 106: 887-95), Takahashi et al (1997, Proc. Natl. Acad. Sci. USA 94: 11102-07) and Koprivova et al. (2000, Plant Physiol. 122: 737-46).

### III.E.2. USE OF RESPIRATION-RESPONSIVE GENES TO MODULATE BIOCHEMICAL ACTIVITIES

The activities of one or more of the respiration responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Respiration and energy-related processes	Mitochondrial Electron Transport Chain	Passam et al. (1973) Biochem Biophys. Acta 325: 54-61
	Alternative oxidase pathway	Saisho et al. (1997) Plant Mol. Biol. 35: 585-600 Vanlerberghe and McIntosh (1994) Plant Physiol. 105: 867-874

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	ATP generation pathways ATP utilization pathways	Mahler and Cordes (1966) In Biological Chemistry, Harper and Row
	Chloroplast energy related pathways	Foyer et al. (1989) Arch. Biochem. Biophys. 268: 687-697 Mills et al. (1978) Biochem. Biophys. Acta 504: 298-309
	Peroxisome energy related pathways	Olsen (1998) Plant mol. Biol. 38: 163-89
	Cytoplasmic energy related pathways	Roberts et al. (1995) Febs Letters 373: 307-309
	Catabolism and Anabolism	Mahler and Cordes (1966) In Biological Chemistry, Harper and Row
	Aerobic versus anaerobic pathways	Mahler and Cordes (1966) In Biological Chemistry, Harper and Row
Coordination of Carbon and Nitrogen Metabolism	Light-regulation of major central carbon and nitrogen Metabolic pathways to coordinate growth	Lam et al. (1998) Plant J. 16(3): 345-353 Lejay et al. (1999) Plant J. 18(5): 509-519; and Oliveira et al. (1999) Plant. Phys. 121: 301-309
	Carbohydrate and nitrogen control of carbohydrate and organic nitrogen accumulation	Lam et al. (1998) Plant J. 16(3): 345-353 Lejay et al. (1999) Plant J. 18(5):

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	pathways	509-519; and Oliveira et al. (1999) Plant. Phys. 121: 301-309

Other biological activities that can be modulated by the respiration genes and gene products are listed in the REF Tables. Assays for detecting such biological activities are described in the Protein Domain table.

Respiration responsive genes are differentially expressed in response to inhibition of mitochondrial electron transport by antimycin A. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table reports the changes in transcript levels of various respiration responsive genes that are differentially expressed in response to this treatment.

Profiles of these different respiration genes are shown in the Table below with examples of associated biological activities.



TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
Up regulated transcripts	<p>Responders to inhibition of mitochondrial electron transport respiration</p> <p>Genes induced by inhibition of mitochondrial electron transport</p>	<ul style="list-style-type: none"> <li>• Changes in generation of ATP</li> <li>• Alternate oxidase induction</li> <li>• Changes in catabolic and anabolic enzymes and pathways</li> <li>• Specific gene transcription initiation</li> <li>• Changes in electron transport proteins</li> </ul>	<ul style="list-style-type: none"> <li>• Transporters</li> <li>• Catabolic and anabolic enzymes</li> <li>• Changes in cell and organelle membrane structures and potentials</li> <li>• Kinases and phosphatases</li> <li>• Transcription activators</li> <li>• Change in chromatin structure and/or localized DNA topology</li> <li>• Redox control</li> </ul>
Down-regulated transcripts	<p>Responders to inhibition of mitochondrial electron transport</p> <p>Genes repressed by inhibition of mitochondrial</p>	<ul style="list-style-type: none"> <li>• Changes in ATP generating pathways</li> <li>• Changes in pathways and processes operating in cells</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription factors</li> <li>• Change in protein structure by phosphorylation (kinases) or dephosphorylation (phosphatases)</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
	electron transport	<ul style="list-style-type: none"> <li>• Induction of aerobic pathways</li> <li>• Changes in catabolism and anabolism</li> </ul>	<ul style="list-style-type: none"> <li>• Transporters</li> <li>• Catabolic and anabolic enzymes</li> <li>• Changes in cell and organelle membrane structures and potentials</li> <li>• Change in chromatin structure and/or localized DNA topology</li> <li>• changes</li> <li>• Stability factors for protein synthesis and degradation</li> <li>• Metabolic enzymes</li> </ul>
		<ul style="list-style-type: none"> <li>• Changes in redox activities</li> </ul>	<ul style="list-style-type: none"> <li>• Changes in redox enzymes</li> </ul>

#### Use of Promoters of Respiration Genes

Promoters of Respiration genes are useful for transcription of any desired polynucleotide or plant or non-plant origin. Further, any desired sequence can be transcribed in a similar temporal, tissue, or environmentally specific patterns as the Respiration genes where the desired

sequence is operably linked to a promoter of a Respiration gene. The protein product of such a polynucleotide is usually synthesized in the same cells, in response to the same stimuli as the protein product of the gene from which the promoter was derived. Such promoter are also useful to produce antisense mRNAs to down-regulate the product of proteins, or to produce sense mRNAs to down-regulate mRNAs via sense suppression.

5

III.F. PROTEIN DEGRADATION GENES, GENE COMPONENTS AND  
PRODUCTS

One of the components of molecular mechanisms that operate to support plant  
development is the "removal" of a gene product from a particular developmental circuit once the  
substrate protein is not functionally relevant anymore in temporal and/or spatial contexts. The  
"removal" mechanisms can be accomplished either by protein inactivation (e.g., phosphorylation  
or protein-protein interaction) or protein degradation most notably via ubiquitination-proteasome  
pathway. The ubiquitination-proteasome pathway is responsible for the degradation of a  
plethora of proteins involved in cell cycle, cell division, transcription, and signal transduction, all  
of which are required for normal cellular functions. Ubiquitination occurs through the activity of  
ubiquitin-activating enzymes (E1), ubiquitin-conjugating enzymes (E2), and ubiquitin-protein  
ligases (E3), which act sequentially to catalyze the attachment of ubiquitin (or other modifying  
molecules that are related to ubiquitin) to substrate proteins (Hochstrasser 2000, Science 289:  
563). Ubiquitinated proteins are then routed to proteasomes for degradation processing [2000,  
Biochemistry and Molecular Biology of Plants, Buchanan, Gruissem, and Russel (eds), Amer.  
Soc. of Plant Physiologists, Rockville, MD]. The degradation mechanism can be selective and  
specific to the concerned target protein (Joazeiro and Hunter 2001, Science 289: 2061; Sakamoto  
et al., 2001, PNAS Online 141230798). This selectivity and specificity may be one of the ways  
that the activity of gene products is modulated.

III.F.1. IDENTIFICATION OF PROTEIN DEGRADATION GENES, GENE  
COMPONENTS AND PRODUCTS

"Protein degradation" genes identified herein are defined as genes, gene components and  
products associated with or dependant on the ubiquitination – proteasome protein degradation  
process. . Examples of such "protein degradation" genes and gene products are shown in the  
Reference and Sequence Tables. The biochemical functions of the protein products of many of  
these genes are also given in the Reference, Sequence, Protein Group, Protein Group Matrix  
tables, MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff and MA\_clust  
tables. Selected genes, gene components and gene products of the invention can be used to  
modulate many plant traits from architecture to yield to stress tolerance.

"Protein Degradation" Genes, Gene Components And Products Identified By Phenotypic

Observations

5 "Protein degradation" genes herein were discovered and characterized from a much larger set of genes in experiments designed to find the genes associated with the increased number of lateral branches (and secondary inflorescences) that are formed per cauline node. In these experiments, "protein degradation" genes were identified using a mutant with these characteristics. The gene causing the changes was identified from the mutant gene carrying an inserted tag. The mutant plant was named 13B12-1 and the mutant was in the E2 conjugating enzyme gene of the ubiquitination process. Compared to "wild-type" parental plants, the mutant plants exhibited multiple lateral stems per node and multi-pistillated flowers. For more experimental detail, see Example section below.

10  
15 Protein Degradation Genes, Gene Components And Products Identified By Differential Expression

"Protein degradation" genes were also identified by measuring the relative levels of mRNA products in the mutant plant 13B12-1 lacking the E2 conjugating enzyme versus a "wild-type" parental plant. Specifically, mRNAs were isolated from 13B12-1 and compared with mRNAs isolated from wild-type plants utilizing microarray procedures. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108451). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

20  
25 Protein Degradation genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

Protein Degradation Genes Identified By Cluster Analyses Of Differential Expression

30 Protein Degradation Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Protein Degradation genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108451 of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

#### Protein Degradation Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Protein Degradation genes. A group in the MA\_clust is considered a Protein Degradation pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

#### Protein Degradation Genes Identified By Amino Acid Sequence Similarity

Protein Degradation genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Protein Degradation genes. Groups of Protein Degradation genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Protein Degradation pathway or network is a group of proteins that also exhibits Protein Degradation functions/utilities.

These differentially expressed genes include genes associated with the degradation process and the genes whose expression is disturbed by the aberrant ubiquitination.

Examples of phenotypes, biochemical activities, and transcription profiles that can be modulated using these genes, gene components and gene products are described above and below.

III.F.2. USE OF "PROTEIN DEGRADATION" GENES, GENE COMPONENTS  
AND PRODUCTS TO MODULATE PHENOTYPES

The "protein degradation" genes, their components and products of the instant invention  
5 are useful for modulating one or more processes required for post-translational modification  
(e.g., ubiquitination) and degradation or inactivation of substrate proteins and also the pathways  
and processes that are associated with protein inactivation that are important for either or all of  
the following: (I) cell proliferation; (II) cell differentiation; and (III) cell death. The "protein  
degradation" genes, gene components and gene products are useful to alter or modulate one or  
10 more of the following phenotypes:

I. Cell Proliferation

A. Cell properties

Cell properties can be critically altered by the maintenance or not of  
regulatory proteins

1. Cell size
2. Cell division, rate and direction
3. Cell elongation

B. Plant size

The following parts of a plant can be modulated by "protein degradation" genes, gene  
components or gene products to affect plant size:

1. Roots

- (a) Primary
- (b) Lateral
- (c) Root hairs
- (d) Root cap
- (e) Apical meristem
- (f) Epidermis
- (g) Cortex

- (h) Stele
- 2. Stem
  - (a) Phloem
  - (b) Xylem
  - (c) Nodes
  - (d) Internodes
  - (e) Leaves
  - (f) Shoot apical meristem
  - (g) Cauline
  - (h) Rosette
  - (i) Petioles
- 3. Flowers
  - (a) Receptacle
  - (b) Sepals, Petals, and Tepals
  - (c) Androecium
  - (d) Stamen
  - (e) Anther
  - (f) Pollen
  - (g) Filament
  - (h) Gynoecium
  - (i) Carpel
  - (j) Ovary
  - (k) Style
  - (l) Stigma
  - (m) Ovule
  - (n) Pedicel and Peduncle
- 4. Seeds
  - (a) Placenta
  - (b) Embryo
  - (c) Cotyledon
  - (d) Endosperm



- (e) Suspensor
- (f) Seed coat (testa)
- 5. Fruits
  - (a) Pericarp – thickness, texture
  - (b) Exocarp
  - (c) Mesocarp
  - (d) Encocarp

## II. CELL DIFFERENTIATION

The intracellular levels of many proteins are regulated by ubiquitin-proteasome proteolysis. Without proper regulation of protein levels, normal cell differentiation can be altered. Examples of cell differentiation and development that are useful to modulated by the genes and gene products of this invention are as follows:

### A. Roots

The polynucleotides and polypeptides of this invention can be used to control root structure and function. Examples are as follows:

- 1. Size
  - (a) Length of primary roots
  - (b) Length of lateral roots
- 2. Function (for more detail see Root section)

### B. Branching and stem formation

- 1. Multiple pistils
- 2. Multiple lateral stems or secondary inflorescence per cauline node
- 3. Internode length

### C. Cell differentiation and/or development in response to hormones

- 1. Auxin

## III. CELL DEATH

Programmed cell death can result from specific and targeted degradation of critical substrate proteins (e.g., transcription factors, enzymes, and proteins involved in signal transduction). Thus, alteration of "protein degradation" genes, their gene products, and the

corresponding substrate proteins that they are acting upon are useful to modulate the vigor and yield of the plant overall as well as distinct cells, organs, or tissues. Examples of traits that can be modulated by these genes and gene products include:

- A. Sterility or reproduction
- B. Seedling lethality

Uses Of Plants That Are Modified As Described Above

Genes that control fundamental steps in regulatory pathways, such as protein inactivation, that in turn influence cascades and networks of other genes and processes are extremely useful. They and their component parts can be used selectively to manipulate development in specific cells, tissues and organs, including meristems when genes are designed to inactivate the normal genes only in specific cells, tissues and organs or to promote protein production where it is not normally produced. They can also be used to promote/control cell death.

Other "protein degradation" genes described here are components of the pathways determining organ identity and phenotypes. These and their component parts are also useful for modifying the characteristics of specific cells, tissues and organs when regulated appropriately. Thus "protein degradation" genes have wide utility for achieving the following:

- A. Better plant survival by decreased lodging
- B. Better responses to high plant density
- C. Better stress tolerance
- D. Better animal (including human) nutrition values
- E. Improved dietary mineral nutrition
- F. More vigor, growth rate and yield
  - 1. Growth rate
    - (a) Whole plant, including height, flowering time, branching, etc.
    - (b) Seedling
    - (c) Coleoptile elongation
    - (d) Young leaves
    - (e) Flowers
    - (f) Seeds
    - (g) Fruit

2. Yield

(a) Biomass

- Fresh and dry weight during any time in plant life, including maturation and senescence

(b) Root/tuber yield

- Number, size, weight, harvest index
- Content and composition, e.g. amino acid, jasmonate, oil, protein and starch

(c) Number of flowers

(d) Seed yield

- Number, size, weight, harvest index
- Content and composition, e.g. amino acid, jasmonate, oil, protein and starch

(e) Fruit yield

- Number, size, weight, harvest index, post harvest quality
- Content and composition, e.g. amino acid, jasmonate, oil, protein and starch

To regulate any of the phenotype(s) above, activities of one or more of the "protein degradation" genes or gene products can be modulated and tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. In addition, a synthetic molecule containing specific domains from "protein degradation" genes or gene product and/or in combination with other domains from gene products that are not necessarily related to protein degradation pathway can be constructed to target the degradation or inactivation of specific substrate proteins. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, *Methods. Mol. Biol.* 82:259-266) and/or screened for variants as in Winkler et al. (1998) *Plant Physiol* 118: 743-50 and visually inspected for the desired phenotype or metabolically and/or functionally assayed according to Dolan et al. (1993, *Development* 119: 71-84), Dolan et al. (1997, *Development* 124: 1789-98), Crawford and Glass (1998, *Trends Plant Science* 3: 389-95), Wang et al. (1998, *PNAS USA* 95: 15134-39), Gaxiola et al. (1998, *PNAS USA* 95: 4046-50), Apse et al. (1999, *Science* 285: 1256-58), Fisher and Long (1992, *Nature*

357: 655-60), Schneider et al. (1998, Genes Devel 12: 2013-21) and Hirsch (1999, Curr Opin Plant Biol. 2: 320-326).

### III.F.3. USE OF PROTEIN DEGRADATION GENES, GENE COMPONENTS AND PRODUCTS TO MODULATE BIOCHEMICAL ACTIVITIES

One or more of the "protein degradation" genes and their components can be used to modulate biochemical or metabolic activities, processes and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Growth, Differentiation and Development	Auxin response	Schwechheimer et al, Science 292: 1379 (2001); Leyser et al, Nature 8: 161 (1993); Lasswell et al, Plant Cell 12: 2395 (2000)
	Photomorphogenesis via leaf cells and meristems	Schwechheimer et al, Science 292: 1379 (2001)
	Apical dominance via shoot meristems	Schwechheimer et al, Science 292: 1379 (2001)
	Lateral root development via root meristem	Xie et al, Genes Dev 14: 3024 (2000)
	Hypocotyl, shoot elongation by hormone controlled process	Nagpal et al, Plant Physiol 123: 563 (2000)
Gene Expression and related cellular processes	mRNA stability	Johnson et al, PNAS 97: 13991 (2000);
	Gene activation	Pham and Sauer, 289: 2357 (2000)
	Cell division and cell cycle control in meristems	King et al, Cell 81: 279 (1995); Ciechanover et al, Cell 37: 57 (1984); Finley et al, Cell 37: 43 (1984);

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
		Robzyk et al, Science 287: 501 (2000)
	Chromatin remodeling	Roest et al, Cell 86: 799 (1996)
	Post-translational modification and organelle targeting of proteins	Biederer et al, Science 278: 1806 (1997)

Other biological activities that can be modulated by the "protein degradation" gene, gene components and products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table.

#### III.F.4. USE OF PROTEIN DEGRADATION GENES, GENE COMPONENTS AND PRODUCTS TO MODULATE TRANSCRIPTION LEVELS OF OTHER GENES

The expression of many genes is "up regulated" or "down regulated" in the 13B12-1 mutant because some protein degradation genes and their products are integrated into complex networks that regulate transcription of many other genes. Some protein degradation genes are therefore useful for modifying the transcription of other genes and hence complex phenotypes, as described above. Profiles of "protein degradation" genes are described in the Table below with associated biological activities. "Up-regulated" profiles are those where the gene produces mRNA levels that are higher in the 13B12-1 as compared to wild-type plant; and vice-versa for "down-regulated" profiles.

TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES WHOSE TRANSCRIPTS ARE CHANGED
Up Regulated Transcripts	<ul style="list-style-type: none"> <li>• Genes induced as a consequence of mutant ubiquitination degradation system</li> <li>• Genes repressed by "protein degradation" system directly or indirectly</li> <li>• Genes repressed or mRNAs degraded as a consequence of mutant ubiquitination degradation process</li> </ul>	<ul style="list-style-type: none"> <li>• Shoot formation</li> <li>• Lateral stem, lateral and main inflorescence development</li> <li>• Internode elongation</li> <li>• Node determination and development</li> <li>• Root formation</li> <li>• Lateral root development</li> <li>• Proper response to Auxin and other growth regulators</li> <li>• Seed dormancy and seed development</li> <li>• Resistance to drought and other forms of stress</li> <li>• Secondary metabolite biosynthesis</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Activators and Repressors</li> <li>• Chromatin Structure and/or Localized DNA Topology determining proteins</li> <li>• Methylated DNA binding proteins</li> <li>• Kinases, Phosphatases</li> <li>• Signal transduction pathway proteins</li> <li>• Transporters</li> <li>• Metabolic Enzymes</li> <li>• Cell cycle checkpoint proteins</li> <li>• Cell Membrane Structure And Proteins</li> <li>• Cell Wall Proteins</li> <li>• Proteins involved in secondary metabolism</li> <li>• Seed storage</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES WHOSE TRANSCRIPTS ARE CHANGED
			metabolism
Down Regulated Transcripts	<ul style="list-style-type: none"> <li>Genes activated by "protein degradation" systems directly or indirectly</li> </ul>		

"Protein degradation" genes and gene products can be modulated alone or in combination as described in the introduction. Of particular interest are combination of these genes and gene products with those that modulate hormone responses and/or metabolism. Hormone responsive and metabolism genes and gene products are described in more detail in the sections above. Such modification can lead to major changes in plant architecture and yield.

#### Use Of Promoters And "Protein Degradation Genes, Gene Components And Products"

Promoters of "protein degradation" genes, as described in the Reference tables, for example, can be used to modulate transcription of any polynucleotide, plant or non plant to achieve synthesis of a protein in association with production of the ubiquitination –proteasome pathway or the various cellular systems associated with it. Additionally such promoters can be used to synthesize antisense RNA copies of any gene to reduce the amount of protein product produced, or to synthesize RNA copies that reduce protein formation by RNA interference. Such modifications can make phenotypic changes and produce altered plants as described above.



III.G. CAROTENOGENESIS RESPONSIVE GENES, GENE COMPONENTS AND  
PRODUCTS

Carotenoids serve important biochemical functions in both plants and animals. In plants, carotenoids function as accessory light harvesting pigments for photosynthesis and to protect chloroplasts and photosystem II from heat and oxidative damage by dissipating energy and scavenging oxygen radicals produced by high light intensities and other oxidative stresses. Decreases in yield frequently occur as a result of light stress and oxidative stress in the normal growth ranges of crop species. In addition light stress limits the geographic range of many crop species. Modest increases in oxidative stress tolerance would greatly improve the performance and growth range of many crop species. The development of genotypes with increased tolerance to light and oxidative stress would provide a more reliable means to minimize crop losses and diminish the use of energy-costly practices to modify the soil environment.

In animals carotenoids such as beta-carotene are essential provitamins required for proper visual development and function. In addition, their antioxidative properties are also thought to provide valuable protection from diseases such as cancer. Modest increases in carotenoid levels in crop species could produce a dramatic effect on plant nutritional quality. The development of genotypes with increased carotenoid content would provide a more reliable and effective nutritional source of Vitamin A and other carotenoid derived antioxidants than through the use of costly nutritional supplements.

Genetic changes produced through DNA mutation in a plant can result in the modulation of many genes and gene products. Examples of such mutation altered genes and gene products are shown in the Reference and Sequence Tables. These genes and/or products are responsible for effects on traits such as plant vigor, nutritional content and seed yield.

While carotenoid synthesis and/or oxidative stress responsive polynucleotides and gene products can act alone, combinations of these polynucleotides also affect growth and development. Useful combinations include different carotenoid biosynthetic polynucleotides and/or gene products that have similar transcription profiles or similar biological activities, and members of the same or similar biochemical pathways. In addition, the combination of an carotenoid synthesis or oxidative stress protective polynucleotide and/or gene product with another environmentally responsive polynucleotide is also useful because of the interactions that exist between hormone regulated pathways, stress pathways, nutritional pathways and

development. Here, in addition to polynucleotides having similar transcription profiles and/or biological activities, useful combinations include polynucleotides that may have different transcription profiles but which participate in a common pathway.

Such carotenoid synthesis/oxidative stress tolerance genes and gene products can  
5 function to either increase or dampen the above phenotypes or activities either in response to changes in light intensity or in the absence of osmotic fluctuations. They were discovered and characterized from a much larger set of genes by experiments designed to find genes whose mRNA products participate in carotenogenesis. These experiments made use of an Arabidopsis mutant (Or) having an accumulation of up to 500 times more beta-carotene than wild-type in  
10 non-photosynthetic tissues.

Microarray technology allows monitoring of gene expression levels for thousands of genes in a single experiment. This is achieved by hybridizing labeled fluorescent cDNA pools to glass slides that contain spots of DNA (Schena et al. (1995) Science 270: 467-70). The  
15 USArabidopsis Functional Genomics Consortium (AFGC) has recently made public the results from such microarray experiments conducted with AFGC chips containing some 10,000 non-redundant ESTs, selected from about 37,000 randomly sequenced ESTs generated from mRNA of different tissues and developmental stages.

The sequences of the ESTs showing at least two-fold increases or decreases in the mutant plant compared with wild type seedlings were identified, compared to the Ceres full length  
20 cDNA and genomic sequence databanks, and equivalent Ceres clones identified. MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table reports the results of this analysis, indicating those Ceres clones which are up or down regulated over controls, thereby indicating the Ceres clones which represent Carotenoid synthesis/oxidative stress tolerance responsive genes. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or  
25 AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: Cauliflower (relating to SMD 5329, SMD 5330)). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Carotenogenesis genes are those sequences that showed differential expression as  
30 compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

5

Carotenogenesis Genes Identified By Cluster Analyses Of Differential Expression

Carotenogenesis Genes Identified By Correlation To Genes That Are  
Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Carotenogenesis genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID Cauliflower (relating to SMD 5329, SMD 5330) of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

Carotenogenesis Genes Identified By Correlation To Genes That Cause  
Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Carotenogenesis genes. A group in the MA\_clust is considered a Carotenogenesis pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

Carotenogenesis Genes Identified By Amino Acid Sequence Similarity

Carotenogenesis genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Carotenogenesis genes. Groups of Carotenogenesis genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a

Carotenogenesis pathway or network is a group of proteins that also exhibits Carotenogenesis functions/utilities.

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III.G.1. USE OF CAROTENOID SYNTHESIS,/OXIDATIVE STRESS  
TOLERANCE RESPONSIVE GENES, GENE COMPONENTS AND  
PRODUCTS TO MODULATE PHENOTYPES

Carotenoid synthesis/oxidative stress tolerance genes and gene products are useful to or modulate one or more of the following phenotypes:

- Growth rate
- Whole Plant, including Height, Flowering Time, etc.
- Seedling
- Organ
  - Stem
  - Leaves
  - Roots
  - Flowers
  - Fruits
  - Seeds
- Yield
- Size, Weight
- Seed Development
- Embryo
- Germination
- Cell Differentiation
- Chloroplasts

- Plant nutrition
- Uptake and assimilation of organic compounds
- Uptake and assimilation of inorganic compounds
- Animal (including human) nutrition
- Improved dietary mineral nutrition
- Stress Responses
- Drought
- Cold
- Osmotic

To improve any of the phenotype(s) above, activities of one or more of the Carotenoid synthesis/oxidative stress tolerance genes or gene products can be modulated and tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266) and/or screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and visually inspected for the desired phenotype or metabolically and/or functionally assayed according to Friedrich, (1999, JAMA 282: 1508), Kumar et al. (1999, Phytochemistry 51: 847-51), La Rocca et al. (2000, Physiologia Plantarum 109: 51-7) and Bartley (1994, In: Ann Rev Plant Physiol Plant Molec Biol, Jones and Somerville, eds, Annual Reviews Inc, Palo Alto, CA).

### III.G.2.USE OF CAROTENOID SYNTHESIS/OXIDATIVE STRESS TOLERANCE RESPONSIVE GENES, GENE COMPONENTS AND PRODUCTS TO MODULATE BIOCHEMICAL ACTIVITIES

The activities of one or more of the carotenoid synthesis/oxidative stress tolerance genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Growth , Differentiation and Development	Chloroplast biosynthesis	Kumar et al. (1999) Phytochemistry 51: 847-51 Fraser et al. (1994) Plant Physiol 105: 405-13
Metabolism	Carotenoid biosynthesis	Kumar et al. (1999) Phytochemistry 51: 847-51
	Herbicide resistance	La Rocca et al. (2000) Physiologia Plantarum 109: 51-57
	Regulate abscisic acid levels	Tan et al. (1997) PNAS USA 94: 12235-40
	Drought, cold and osmotic tolerance	Tan et al. (1997) PNAS USA 94: 12235-40

Other biological activities that can be modulated by the Carotenoid synthesis, oxidative stress tolerance genes and gene products are listed in the Reference Tables. Assays for detecting such biological activities are described in the Protein Domain table.

5 Profiles of these different carotenoid synthesis/oxidative stress tolerance responsive genes are shown in the Table below together with examples of the kinds of associated biological activities.

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
Up regulated transcripts	Genes induced during carotenoid synthesis/oxidative stress tolerance activity	<ul style="list-style-type: none"> <li>• Gene Repression/Induction activity</li> <li>• Cell cycle progression</li> <li>• Chromatin condensation</li> <li>• Synthesis of metabolites and/or proteins</li> <li>• Modulation of transduction pathways</li> <li>• Specific gene transcription initiation</li> </ul>	<ul style="list-style-type: none"> <li>• Transporters</li> <li>• Metabolic enzymes</li> <li>• Kinases and phosphatases</li> <li>• Transcription activators</li> <li>• Change in chromatin structure and/or localized DNA topology</li> </ul>
Down-regulated transcripts	<p>Genes repressed during carotenoid synthesis/oxidative stress tolerance activity</p> <p>Genes with discontinued expression or unstable mRNA in conditions of reduced carotenoid synthesis/oxidative stress tolerance</p>	<ul style="list-style-type: none"> <li>• Gene repression/induction activity</li> <li>• Changes in pathways and processes operating in cells</li> <li>• Changes in metabolism other than carotenoid synthesis/oxidative stress tolerance</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription factors</li> <li>• Change in protein structure by phosphorylation (kinases) or dephosphorylation (phosphatases)</li> <li>• Change in chromatin structure and/or DNA topology</li> <li>• Stability of</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
			factors for protein synthesis and degradation • Metabolic enzymes

#### Use of Promoters of Carotenogenesis Responsive Genes

Promoters of Carotenogenesis responsive genes are useful for transcription of any desired polynucleotide or plant or non-plant origin. Further, any desired sequence can be transcribed in a similar temporal, tissue, or environmentally specific patterns as the Carotenogenesis responsive genes where the desired sequence is operably linked to a promoter of a Carotenogenesis responsive gene. The protein product of such a polynucleotide is usually synthesized in the same cells, in response to the same stimuli as the protein product of the gene from which the promoter was derived. Such promoter are also useful to produce antisense mRNAs to down-regulate the product of proteins, or to produce sense mRNAs to down-regulate mRNAs via sense suppression.



IV. VIABILITY GENES, GENE COMPONENTS AND PRODUCTS

IV.A. VIABILITY GENES, GENE COMPONENTS AND PRODUCTS

Plants contain many proteins and pathways that when blocked or induced lead to cell,  
organ or whole plant death. Gene variants that influence these pathways can have profound  
effects on plant survival, vigor and performance. The critical pathways include those concerned  
with metabolism and development or protection against stresses, diseases and pests. They also  
include those involved in apoptosis and necrosis. The applicants have elucidated many such  
genes and pathways by discovering genes that when inactivated lead to cell or plant death.

Herbicides are, by definition, chemicals that cause death of tissues, organs and whole  
plants. The genes and pathways that are activated or inactivated by herbicides include those that  
cause cell death as well as those that function to provide protection. The applicants have  
elucidated these genes.

The genes defined in this section have many uses including manipulating which cells,  
tissues and organs are selectively killed, which are protected, making plants resistant to  
herbicides, discovering new herbicides and making plants resistant to various stresses.

IV.A.1. IDENTIFICATION OF VIABILITY GENES, GENE COMPONENTS  
AND PRODUCTS

Viability genes identified here are defined as genes, gene components and products  
capable of inhibiting cell, tissue, organ or whole plant death or protecting cells, organs and plants  
against death and toxic chemicals or stresses. Examples of such viability genes and gene  
products are shown in the Reference, Sequence, Protein Group, Protein Group Matrix tables,  
MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff, MA\_clust, Knock-in  
and Knock-out tables. The biochemical functions of the protein products of many of these genes  
determined from comparisons with known proteins are also given in the Reference tables.

Viability Genes, Gene Components And Products Identified By Phenotypic Observations

These genes were discovered and characterized from a much larger set of genes by  
experiments designed to find genes that cause serious disturbances in progeny survival, seed  
germination, development, embryo and/or seedling growth. In these experiments, viability genes

were identified by either (1) ectopic expression of a cDNA in a plant or (2) mutagenesis of a plant genome. The plants were then cultivated and one or more of the following phenotypes, which varied from the parental wild-type was observed:

- A. Gametophytic loss of progeny seedlings (detected from a parent on the basis of a linked herbicide resistance gene showing abnormal segregation ratios, as revealed by treating with herbicide)
- B. Embryo death, resulting in some cases to loss of seed
- C. Pigment variation in cotyledons and leaves, including absence of chlorophyll, which leads to seedling death.
  - 1. Abinos
  - 2. Yellow/greens
- D. Cotyledons produced but no or few leaves and followed by seedling death.
- E. Very small plantlets

The genes identified in these experiments are shown in Tables X.

Viability Genes, Gene Components And Products Identified By Differential Expression

Viability genes were also identified from a much larger set of genes by experiments designed to find genes whose mRNA products changed in concentration in response to applications of different herbicides to plants. Viability genes are characteristically differentially transcribed in response to fluctuating herbicide levels or concentrations, whether internal or external to an organism or cell. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table reports the changes in transcript levels of various viability genes in entire seedlings at 0, 4, 8, 12, 24, and 48 hours after a plant was sprayed with a Hoagland's nutrient solution enriched with either 2,4 D (Trimec), Glean, Grassgetter, Roundup, or Finale herbicides as compared to seedlings sprayed with Hoagland's solution only.

The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108467, 107871, 107876, 108468, 107881, 108465, 107896, 108466, 107886, 107891, 108501). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example

section below.

Viability genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

5

Viability Genes Identified By Cluster Analyses Of Differential Expression

Viability Genes Identified By Correlation To Genes That Are Differentially

Expressed

As described above, the transcription profiles of genes that act together are well correlated.

10 Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

15 A pathway or network of Viability genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108467, 107871, 107876, 108468, 107881, 108465, 107896, 108466, 107886, 107891, 108501 of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

Viability Genes Identified By Correlation To Genes That Cause

20 Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Viability genes. A group in the MA\_clust is considered a Viability pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

25

Viability Genes Identified By Amino Acid Sequence Similarity

Viability genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Viability genes. Groups of Viability genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Viability pathway or network is a group of proteins that also exhibits Viability functions/utilities.

30

It is assumed that those gene activity changes in response to the toxic herbicides are either responsible, directly or indirectly, for cell death or reflect activation of defense pathways. These genes are therefore useful for controlling plant viability.

Examples of phenotypes, biochemical activities, or transcript profiles that can be modulated using selected viability gene components are described above and below.

#### IV.A.2.USE OF VIABILITY GENES, GENE COMPONENTS AND PRODUCTS TO MODULATE PHENOTYPES

Deficiencies in viability genes can cause cell death at various rates and under various conditions. Viability genes can be divided into two classes; (1) those that lead to cell death under permissive growth conditions and (2) those that cause cell demise under restrictive conditions. Examples of the first class are viability genes which encode toxins or which participate in the programmed cell death pathway(s). Disruption of metabolic pathways, such as amino acid synthesis, may not cause death when the cell is supplemented with appropriate amino acids, but can cause death under more restrictive conditions.

Some deficiencies in viability genes identified cause the organism as a whole to die, while other genes cause death only of a specific subset of cells or organs. For example, genes identified from embryo viability phenotypes can cause an entire organism to die. In contrast, genes characterized from gametophytic lethals may inhibit cell growth only in a select set of cells. In addition, some viability genes may not cause an immediate demise. A seedling lethal phenotype is one such example, where a seed germinates and produces cotyledons but the plant dies before producing any true leaves. Yellow-green pigment mutants provide yet another set of examples. In some cases, the plant produces a number of yellow-green leaves but dies before producing any seed, due in part, to the necessity to produce chlorophyll in functioning chloroplasts to fix CO<sub>2</sub>.

Viability genes, in which mutational deficiencies lead to death, carry no duplicates in the haploid plant genome. They thus may be especially likely to promote viability and vigor when expressed more optimally in a plant, in specific tissues or throughout the plant.

Proteins which lead to death when inactivated, and other proteins in the pathways in which they act, are potential targets for herbicides. In this kind of application, chemicals specifically capable of interacting with such proteins are discovered. Typically, this could be done by designing a gene involving the relevant viability gene, that also facilitates a rapid easily measured assay for the functioning of the protein product, and treating plants containing the new genes with the potential herbicides. Those chemicals specifically interfering with the protein activity can then easily be selected for further development.

Genes whose products interact directly with a herbicide can also be modified such that the herbicide no longer inactivates the protein. Such genes are useful for making herbicide resistant plants, valuable in agriculture.

Many of the genes activated or inactivated by the herbicides define genes involved in the pathways that protect the plant against damage and stresses. These genes and gene components, especially those regulating such pathways, are especially useful for enhancing the ability of plants to withstand specific stresses, including herbicides. [See the sections on Stress responsive genes, gene components and products.]

Genes that cause cellular death can be used to design new genes that cause death of specific cells and tissues and hence new valuable products. For example, activation of genes causing death in cells specifying seeds can be used to produce fruits lacking seeds. They can also be used to prevent cell death by pathogens and pests.

The genes and gene components of the instant invention are useful to modulate one or more processes that affect viability and vigor at the (I) cellular level; (II) organelle level; (III) organ level; or (IV) overall organism level.

Examples of phenotypes that are modulated are described above and below:

I. CELLULAR LEVEL:

Viability genes and gene products are useful in modulating cellular changes in:

- A. Cell size
- B. Cell differentiation
- C. Cell division
- D. Cell longevity
- E. Cell position

F. Cytotoxins

II. ORGANELLE LEVEL

The development, growth, and viability of chloroplasts and/or mitochondria can be  
5 modulated by the genes and gene products of the instant invention:

III. ORGAN LEVEL:

The invention is also useful to regulate the development, growth, and viability of the  
following organs:

- 10 A. Flower 1. number  
2. size
- B. Seed  
1. Size  
Number  
Composition  
- Amino Acid, carbohydrates, lipid, and secondary metabolites
- 15 C. Fruit  
1. Size  
2. Number  
20 3. Composition  
- Amino Acid, carbohydrates, lipid, and secondary metabolites  
1. Fruit Drop  
2. Fruit Ripening
- 25 D. Leaf  
1. Size  
2. Composition  
3. Amino acid, carbohydrates, lipid, and secondary metabolites  
4. Photoefficiency  
5. Abscission  
30 6. Senescence
- E. Stem

F. Root

IV. OVERALL ORGANISM LEVEL

The following traits can be modulated with the genes, gene components and gene products of this invention to affect the strength of a plant as a whole:

- A. Vigor
  - Increased biomass
- B. Stress Tolerance
  - 1. Cold
  - 2. Drought
  - 3. Heat
  - 4. Herbicide
  - 5. Oxidative
  - 6. Salt
- C. Pathogen Resistance

To regulate any of the phenotype(s) above, activities of one or more of the viability genes or gene products can be modulated in an organism and tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (Methods. Mol. Biol. 82:259-266 (1998)) and/or screened for variants as in Winkler et al., Plant Physiol. 118: 743-50 and visually inspected for the desired phenotype or metabolically and/or functionally assayed.

IV.A.3. USE OF VIABILITY GENES, GENE COMPONENTS AND PRODUCTS  
TO MODULATE BIOCHEMICAL ACTIVITIES

The viability genes, their components and/or products can be used to modulate processes, biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Amino Acid Synthesis	Aceto -lactate synthase	Hershey et al. (1999) Plant Mol. Biol. 40, 795-806
Cell Wall Synthesis	Cellulose synthase	Peng et al. (2001) Plant Physiol. 126, 981-982 Kawagoe and Delmer (1997) Genet Eng. 19, 63-87
Nucleotide Synthesis	Coenzyme A biosynthesis	Kupke et al. (2001) J. Biol. Chem. 276, 19190-19196
Lipid Synthesis	Oleosin biosynthesis	Singh et al. (2000) Biochem. Soc. Trans. 28, 925-927 Zou et al. (1996). Plant Mol. Biol. 31, 429-433
Hormone Signaling Pathways	Brassinolide and light signal transduction	Kang et al. (2001) Cell 105, 625-636
Hormone Biosynthesis	Cytokinin biosynthesis	Takei et al, (2001) J. Biol. Chem. 276, 26405-26410
Secondary Metabolites	• Carotenoid biosynthesis	Estevez et al. (2001) J. Biol. Chem. 276, 22901-22909 Carol and Kuntz (2001) Trendy Plant Sci. 6, 31-36 Pogson and Rissler (2001) Phil. Trans. Roy. Soc. Lord. B 355, 1395-1400
Clearing of Toxic Substances	Ubiquitination	
Growth, Differentiation	• Farnesylation	Pei et al (1998) Science 282:



PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
And Development	<ul style="list-style-type: none"> <li>Nitrogen Metabolism</li> </ul>	287-290; Cutler et al. (1996) Science 273: 1239 Goupil et al (1998) J Exptl Botany 49:1855-62
Water Conservation And Resistance To Drought And Other Related Stresses	<ul style="list-style-type: none"> <li>Stomatal Development And Physiology</li> <li>Stress Response Pathways</li> <li>Inhibition Of Ethylene Production Under Low Water Potential</li> <li>Proline And Other Osmolite Synthesis And Degradation</li> </ul>	Allen et al. (1999) Plant Cell 11: 1785-1798 Li et al. 2000 Science 287: 300-303 Burnett Et Al 2000. J. Exptl Botany 51: 197-205 Raschke (1987) In: Stomatal Function Zeiger et al. Eds., 253-279 Bush And Pages (1998) Plant Mol. Biol. 37: 425-35 Spollen Et Al (2000) Plant Physiol. 122:967-976  Hare et al. (1998) Plant, Cell And Environment 21:535- 553; Hare et al. (1999) J. Exptl. Botany 50:413-434
Programmed cell death	<ul style="list-style-type: none"> <li>Proteases</li> <li>DNA endonucleases</li> <li>Mitochondriae uncoupling proteins</li> </ul>	Kamens et al. (1995) J. Biol. Chem. 270, 15250-15256 Wang et al. (2001) Anticancer Res. 21, 1789- 1794 Drake et al. (1996) Plant

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
		<p>Mol. Biol 304, 755-767 Mittler and Lam (1995) Plant Cell 7, 1951-1962 Mittler and Lam (1995) Plant Physiol. 108, 489-493 Thelen and Northcote (1989) Planta 179, 181-195 Hanak and Jezek (2001) FEBS Lett. 495, 137-141</p>
	<ul style="list-style-type: none"> <li>• Plasmalemma and Tonoplast Ion Channel Changes</li> <li>• Ca<sup>2+</sup> Accumulation</li> <li>• K<sup>+</sup> Efflux</li> <li>• Activation Of Kinases And Phosphatases</li> </ul>	<p>Macrobbie (1998) Philos Trans R Soc Lond B Biol Sci 353: 1475-88; Li et al (2000) Science 287:300- 303; Barkla et al. (1999) Plant Physiol. 120:811-819 Lacombe et al. (2000) Plant Cell 12: 837-51; Wang et al. (1998) Plant Physiol 118:1421-1429; Shi et al. (1999) Plant Cell 11: 2393- 2406 Gaymard et al. (1998) Cell 94:647-655 Jonak et al. (1996) Proc. Natl. Acad. Sci 93: 11274- 79; Sheen (1998) Proc. Natl. Acad. Sci. 95: 975-80; Allen</p>

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
		et al. (1999) Plant Cell 11: 1785-98

Other biological activities that can be modulated by the viability genes, their components and products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table.

#### IV.A.4. USE OF VIABILITY GENES, GENE COMPONENTS AND PRODUCTS TO MODULATE TRANSCRIPT LEVELS OF OTHER GENES

The expression of many genes is “up regulated” or “down regulated” following herbicide treatment and also in the leaf mutants, because some “viability” genes and their products are integrated into complex networks that regulate transcription of many other genes. Some “viability genes” are therefore useful for modifying the transcription of other genes and hence complex phenotypes, as described above. The data from differential expression experiments can be used to identify a number of types of transcript profiles of “viability genes”, including “early responders,” and “delayed responders”, “early responder repressors” and “delayed repressors”. Profiles of these different types responsive genes are shown in the Table below together with examples of the kinds of associated biological activities. “Up-regulated” profiles are those where the mRNA transcript levels are higher in the herbicide treated plants as compared to the untreated plants. “Down-regulated” profiles represent higher transcript levels in the untreated plant as compared to the herbicide treated plants.

TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES WHOSE TRANSCRIPTS ARE CHANGED

TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES WHOSE TRANSCRIPTS ARE CHANGED
Up Regulated Transcripts (Level At 4 Hr $\cong$ 0 Hr) or (Level At 4 Hr > 0 Hr)	<ul style="list-style-type: none"> <li>• Early Responders To:               <ul style="list-style-type: none"> <li>• Gluphosinate</li> <li>• Chlorsulfuron</li> <li>• Glyphosate and/or 2, 4-D</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Suppression of cell, tissue, organ or plant death following:               <ul style="list-style-type: none"> <li>• Herbicide treatment or under stress</li> </ul> </li> <li>• Activation of cell, tissue, organ or plant death following:               <ul style="list-style-type: none"> <li>• Herbicide treatment or under stress</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> <li>• Transporters</li> <li>• Change In Cell Membrane Structure</li> <li>• Kinases And Phosphatases</li> <li>• Germins, Germin-like proteins, Calcium-binding proteins and H<sub>2</sub>O<sub>2</sub> generating and H<sub>2</sub>O<sub>2</sub> neutralizing proteins.</li> <li>• Transcription Activators</li> <li>• Change In Chromatin Structure And/Or Localized DNA Topology</li> <li>• Annexins, cell wall structural proteins</li> </ul>
Up Regulated Transcripts	Delayed Responders to Gluphosinate,	<ul style="list-style-type: none"> <li>• Suppression of cell, tissue, organ</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES WHOSE TRANSCRIPTS ARE CHANGED
(Level At 4 Hr < 12 Hr)	Chlorsulfuron, Glyphosate and/or 2, 4-D	<p>or plant death following:</p> <ul style="list-style-type: none"> <li>• Herbicide treatment or under stress</li> <li>• Activation of cell, tissue, organ or plant death following: <ul style="list-style-type: none"> <li>• Herbicide treatment or under stress</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Specific Factors (Initiation And Elongation) For Protein Synthesis</li> <li>• Lipid transfer proteins</li> <li>• Myrosinase-binding proteins</li> <li>• Sugar interconverting enzymes</li> <li>• Maintenance Of mRNA Stability</li> <li>• Maintenance Of Protein Stability</li> <li>• Maintenance Of Protein-Protein Interaction</li> <li>• Protein translocation factors</li> <li>• RNA-binding proteins</li> <li>• Centromere and cytoskeleton proteins</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES WHOSE TRANSCRIPTS ARE CHANGED
			<ul style="list-style-type: none"> <li>• Lipases</li> <li>• Zn/Cu transporters</li> <li>• Cell wall structural proteins</li> </ul>
Down-Regulated Transcripts (Level At 0 Hr $\cong$ 4 Hr) or (Level At 0 Hr > 4 Hr)	Early Responder Repressors Of Stress Response State Of Metabolism  Genes With Discontinued Expression Or Unstable mRNA In Presence Of Herbicide or Abiotic Stress	<ul style="list-style-type: none"> <li>• Suppression of cell, tissue, organ or plant death following: <ul style="list-style-type: none"> <li>• Herbicide treatment or under stress</li> </ul> </li> <li>• Activation of cell, tissue, organ or plant death following: <ul style="list-style-type: none"> <li>• Herbicide treatment or under stress</li> <li>• Zn/Cu transporters</li> <li>• Cell wall structural proteins</li> </ul> </li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> <li>• Change In Protein Structure By Phosphorylation (Kinases) Or Dephosphorylation (Phosphatases)</li> <li>• Change In Chromatin Structure And/Or DNA Topology</li> <li>• H<sub>2</sub>O<sub>2</sub> neutralizing proteins</li> <li>• Neutralizing proteins including SOD and GST</li> </ul>
Down-Regulated Transcripts	Delayed Responder Repressors Of ABA	<ul style="list-style-type: none"> <li>• Suppression of cell, tissue, organ</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES WHOSE TRANSCRIPTS ARE CHANGED	PHYSIOLOGICAL CONSEQUENCES OF MODIFYING GENE PRODUCT LEVELS	EXAMPLES OF BIOCHEMICAL ACTIVITIES WHOSE TRANSCRIPTS ARE CHANGED
(Level At 4 Hr > 12 Hr)	Function State Of Metabolism	or plant death following:	<ul style="list-style-type: none"> <li>• Kinases And Phosphatases</li> <li>• Stability Of Factors For Protein Synthesis And Degradation</li> <li>• Amino Acid biosynthesis proteins including asparagine synthase</li> </ul>
	Genes With Discontinued	<ul style="list-style-type: none"> <li>• Herbicide treatment or under stress</li> <li>• Activation of cell, tissue, organ or plant death following:</li> <li>• Herbicide treatment or under stress</li> </ul>	
	Expression Or Unstable mRNA In Presence Of herbicide or Abiotic Stress		<ul style="list-style-type: none"> <li>• Ca-binding proteins</li> <li>• Lipid biosynthesis proteins</li> <li>• Lipases</li> <li>• Zn/Cu transporters</li> <li>• Cell wall structural proteins</li> </ul>

While viability modulating polynucleotides and gene products can act alone, combinations of these polynucleotides also affect growth and development.

IV.A.5. USE OF PROMOTERS OF VIABILITY GENES, GENE COMPONENTS  
AND PRODUCTS

Promoters of viability genes can include those that are induced by (1) destructive chemicals, e.g. herbicides, (2) stress, or (3) death. These promoters can be linked operably to achieve expression of any polynucleotide from any organism. Specific promoters from viability genes can be selected to ensure transcription in the desired tissue or organ. Proteins expressed under the control of such promoters can include those that can induce or accelerate death or those that can protect plant cells organ death. For example, stress tolerance can be increased by using promoters of viability genes to drive transcription of cold tolerance proteins, for example. Alternatively, promoters induced by apoptosis can be utilized to drive transcription of antisense constructs that inhibit cell death.



IV.B. HISTONE DEACETYLASE (AXEL) RESPONSIVE GENES, GENE  
COMPONENTS AND PRODUCTS

5 The deacetylation of histones is known to play an important role in regulating gene  
expression at the chromatin level in eukaryotic cells. Histone deacetylation is catalyzed by  
proteins known as histone deacetylases (HDACs). HDACs are found in multisubunit complexes  
that are recruited to specific sites on nuclear DNA thereby affecting chromatin architecture and  
target gene transcription. Mutations in plant HDAC genes cause alterations in vegetative and  
reproductive growth that result from changes in the expression and activities of HDAC target  
genes or genes whose expression is governed by HDAC target genes. For example, transcription  
factor proteins control whole pathways or segments of pathways and proteins also control the  
activity of signal transduction pathways. Therefore, manipulation of these types of protein levels  
is especially useful for altering phenotypes and biochemical activities.

10 Manipulation of one or more HDAC gene activities are useful to modulate the biological  
activities and/or phenotypes listed below. HDAC genes and gene products can act alone or in  
combination. Useful combinations include HDAC genes and/or gene products with similar  
biological activities, or members of the same, co-regulated or functionally related biochemical  
pathways. Such HDAC genes and gene products can function to either increase or dampen these  
phenotypes or activities.

15 Examples of genes whose expression is affected by alterations in HDAC activity are  
shown in the Reference and Sequence Tables. These genes and/or gene products are responsible  
for effects on traits such as inflorescence branching and seed production. They were discovered  
and characterized from a much larger set of genes by experiments designed to find genes whose  
mRNA products are affected by a decrease in HDAC gene activity. These experiments made use  
of an Arabidopsis mutant having severely reduced mRNA levels for the histone deactylase gene  
AtHDAC1.

20 Microarray technology allows monitoring of gene expression levels for thousands  
of genes in a single experiment. This is achieved by simultaneously hybridizing two  
differentially labeled fluorescent cDNA pools to glass slides that contain spots of DNA (Schen  
et al. (1995) Science 270: 467-70). The Arabidopsis Functional Genomics Consortium (AFGC)  
has recently made public the results from such microarray experiments conducted with AFGC

chips containing 10,000 non-redundant ESTs, selected from 37,000 randomly sequenced ESTs generated from mRNA of different tissues and developmental stages.

The sequences of the ESTs showing at least two-fold increases or decreases over the controls were identified, compared to the Ceres full-length cDNA and genomic sequence databanks, and identical Ceres clones identified. MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table reports the results of this analysis, indicating those Ceres clones which are up or down regulated over controls, thereby indicating the Ceres clones which are HDAc genes. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: Axel (relating to SMD 6654, SMD 6655)). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Histone Deacetylase genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### Histone Deacetylase Genes Identified By Cluster Analyses Of Differential Expression

##### Histone Deacetylase Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Histone Deacetylase genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID Axel (relating to SMD 6654, SMD 6655) of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

##### Histone Deacetylase Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Histone Deacetylase genes. A group in the MA\_clust is considered a Histone Deacetylase pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

#### Histone Deacetylase Genes Identified By Amino Acid Sequence Similarity

Histone Deacetylase genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Histone Deacetylase genes. Groups of Histone Deacetylase genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Histone Deacetylase pathway or network is a group of proteins that also exhibits Histone Deacetylase functions/utilities.

#### IV.B.1.USE OF HDAC GENES, GENE COMPONENTS AND PRODUCTS TO MODULATE PHENOTYPES

HDAC genes and gene products are useful to or modulate one or more of the following phenotypes:

- Growth Rate
- Whole Plant, Including Height, Flowering Time, Etc.
- Seedling
- Organ
  - Stem
  - Leaves
  - Roots
  - Flowers
  - Fruits
  - Seeds
  - Yield

- Size, Weight
- Seed Development
- Embryo
- Germination
- Cell Differentiation

To improve any of the phenotype(s) above, activities of one or more of the HDAC genes or gene products can be modulated and tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266) and visually inspected for the desired phenotype or metabolically and/or functionally assayed according to Wu et al. (2000, Plant J 22: 19-27), Hu et al. (2000, J Biol Chem 275: 15254-64), Johnson and Turner (1999, Semin Cell Dev Biol 10: 179-88), Koyama et al. (2000, Blood 96: 1490-5), Wu et al. (2000, Plant J 22: 19-27), Li (1999, Nature Genetics 23: 5-6), Adams et al. (2000, Development 127: 2493-2502) and Lechner et al. (2000, Biochemistry 39: 1683-92).

#### IV.B.2.USE OF HDAC DEVELOPMENT GENES, GENE COMPONENTS AND PRODUCTS TO MODULATE BIOCHEMICAL ACTIVITIES

The activities of one or more of the HDAC genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Growth , Differentiation And Development	<ul style="list-style-type: none"> <li>• Cell Differentiation</li> <li>• Cell Cycle Progression</li> </ul>	<p>Koyama et al. (2000) Blood 96: 1490-5</p> <p>Hu et al. (2000) J Biol Chem 275: 15254-64</p>
Metabolism	<ul style="list-style-type: none"> <li>• Chromatin Structure</li> </ul>	<p>Hu et al. (2000) J Biol Chem 275: 15254-64</p>

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	<ul style="list-style-type: none"> <li>Gene Transcription And Chromatin Assembly</li> </ul>	Johnson and Turner (1999) Semin Cell Dev Biol 10: 179-88
Reproduction And Seed Development	<ul style="list-style-type: none"> <li>Seed Development</li> <li>Seed Germination</li> <li>Independent Embryo Fertilization</li> <li>Fertilization Independent Seed Development</li> <li>Megagametogenesis</li> </ul>	Wu et al. (2000) Plant J 22:19-27  Lechner et al. (2000) Biochemistry 39: 1683-92 Ohad et al. (1996) PNAS USA 93: 5319-24  Chaudhury et al. (1997) PNAS USA 94: 4222-28  Christensen et al. (1997) Sex Plant Reproduc 10: 49-64

Other biological activities that can be modulated by the HDAC genes and gene products are listed in the REFERENCE Table. Assays for detecting such biological activities are described in the Protein Domain table.

- 5 Profiles of these different HDAC genes are shown in the Table below with examples of associated biological activities.

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
Up Regulated Transcripts	Responders To HDAC Activity	<ul style="list-style-type: none"> <li>• Gene Repression Activity</li> <li>• Cell Cycle Progression</li> <li>• Chromatin Condensation</li> <li>• Synthesis Of Metabolites And/Or Proteins</li> <li>• Modulation Of Transduction Pathways</li> <li>• Specific Gene Transcription Initiation</li> </ul>	<ul style="list-style-type: none"> <li>• Transporters</li> <li>• Metabolic enzymes</li> <li>• Kinases and phosphatases</li> <li>• Transcription activators</li> <li>• Change in chromatin structure and/or localized DNA topology</li> </ul>
Down-Regulated Transcripts	<p>Responder To Hdac Inhibitors</p> <p>Genes With Discontinued Expression Or Unstable Mrna In Presence Of Hdac</p>	<ul style="list-style-type: none"> <li>• Negative Regulation Of Acetylation Pathways</li> <li>• Changes In Pathways And Processes Operating In Cells</li> <li>• Changes In</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription factors</li> <li>• Change in protein structure by phosphorylation (kinases) or dephosphorylation (phosphatases)</li> <li>• Change in chromatin structure</li> </ul>

TRANSCRIPT LEVELS	TYPE OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
		Metabolism	and/or DNA topology <ul style="list-style-type: none"> <li>• Stability of factors for protein synthesis and degradation</li> <li>• Metabolic enzymes</li> </ul>

#### Use of Promoters of Histone Deacetylase Responsive Genes

Promoters of Histone Deacetylase responsive genes are useful for transcription of any desired polynucleotide or plant or non-plant origin. Further, any desired sequence can be transcribed in a similar temporal, tissue, or environmentally specific patterns as the Histone Deacetylase responsive genes where the desired sequence is operably linked to a promoter of a Histone Deacetylase responsive gene. The protein product of such a polynucleotide is usually synthesized in the same cells, in response to the same stimuli as the protein product of the gene from which the promoter was derived. Such promoter are also useful to produce antisense mRNAs to down-regulate the product of proteins, or to produce sense mRNAs to down-regulate mRNAs via sense suppression.

V. STRESS RESPONSIVE GENES, GENE COMPONENTS AND PRODUCTS

V.A. COLD RESPONSIVE GENES, GENE COMPONENTS AND PRODUCTS

5 The ability to endure low temperatures and freezing is a major determinant of the geographical distribution and productivity of agricultural crops. Even in areas considered suitable for the cultivation of a given species or cultivar, can give rise to yield decreases and crop failures as a result of aberrant, freezing temperatures. Even modest increases (1-2°C) in the freezing tolerance of certain crop species would have a dramatic impact on agricultural productivity in some areas. The development of genotypes with increased freezing tolerance would provide a more reliable means to minimize crop losses and diminish the use of energy-costly practices to modify the microclimate.

10 Sudden cold temperatures result in modulation of many genes and gene products, including promoters. Examples of such cold responsive genes and gene products are shown in the Reference, Sequence, Protein Group, Protein Group Matrix tables, MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff and MA\_clust tables. These genes and/or products are responsible for effects on traits such as plant vigor and seed yield. They were discovered and characterized from a much larger set by experiments designed to find genes whose mRNA products changed in response to cold treatment.

20 Manipulation of one or more cold responsive gene activities are useful to modulate the biological activities and/or phenotypes listed below. Cold responsive genes and gene products can act alone or in combination. Useful combinations include cold responsive genes and/or gene products with similar transcription profiles, similar biological activities, or members of the same or functionally related biochemical pathways. Whole pathways or segments of pathways are controlled by transcription factor proteins and proteins controlling the activity of signal transduction pathways. Therefore, manipulation of the levels of such proteins is especially useful for altering phenotypes and biochemical activities of plants. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108578, 108579, 108533, 108534 ). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript



levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Cold genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### Cold Genes Identified By Cluster Analyses Of Differential Expression

##### Cold Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Cold genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108578, 108579, 108533, 108534 of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

##### Cold Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Cold genes. A group in the MA\_clust is considered a Cold pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

#### Cold Genes Identified By Amino Acid Sequence Similarity

Cold genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Cold genes. Groups of Cold genes are identified in the Protein Group table. In this table, any protein group that

comprises a peptide ID that corresponds to a cDNA ID member of a Cold pathway or network is a group of proteins that also exhibits Cold functions/utilities.

Such cold responsive genes and their products can function to either increase or dampen the phenotypes and activities below either in response to cold treatment or in the absence of cold temperature fluctuations.

Further, promoters of cold responsive genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by ABA or any of the following phenotypes or biological activities below.

#### V.A.1. USE OF COLD-RESPONSIVE GENES TO MODULATE PHENOTYPES

Cold responsive genes and gene products are useful to or modulate one or more of the following phenotypes:

- Cold Tolerance, below 7°C, for example
- Cells
- Organelles
- Proteins
- Dehydration Resistance
- Growth rate
- Whole Plant, including height, bolting time, etc.
- Organs
  - Roots
  - Lateral Roots
  - Leaves
  - Stems
  - Flowers
  - Fruit
- Biomass
- Fresh and Dry Weight during any time in plant life, such as maturation;
- Number, Size, and/or Weight
  - Flowers;

- Seeds;
- Branches;
- Leaves;
- Seed Yield
- Number, Size, Weight, Harvest Index, Water Content
- Fruit Yield
- Number, Size, Weight, Harvest Index, Water Content

To regulate any of the phenotype(s) above, activities of one or more of the cold responsive genes or gene products can be modulated and the plants can be tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and assayed, for example, in accordance to Steponokus et al. (1993) Biochimica et Biophysica Acta 1145: 93-104; Quinn (1988) Symp Soc. Exp. Biol. 42: 237-258; Bectold and Pelletier (1998) Methods Mol. Biol. 82: 259-266; Kasuga et al. (1999) Nature Biotechnology 17: 287-291; Guy et al. (1998) Cryobiology 36: 301-314; or Liu et al. (1998) Plant Cell 10: 1391-1406.

#### V.A.2. USE OF COLD-RESPONSIVE GENES TO MODULATE BIOCHEMICAL ACTIVITIES

The activities of one or more of the cold responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities are documented and can be measured according to the citations above and those included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Cold Tolerance	Viability Of Plant Protoplasts At Low Temperatures.	Steponkus (1998) PNAS USA 95: 14570-14575
	Viability Of Yeast At Low Temperatures.	Schirmer et al. (1994) Plant Cell 6: 1899-1909
	Complementation Of Yeast Tsp Mutant	Zentella et al. (1999) Plant Physiology, 119: 1473-1482
	Viability Of E.Coli At Low Temperatures.	Yeh et.al. (1997) PNAS 94: 10967-10972
	Induction Of Cold Shock Response Genes	Pearce (1999) Plant Growth Regulation 29: 47-76.
Lipid Composition	Altered Composition Of Membrane Fatty Acids	Sayanova et al. (1999) Journal of Experimental Botany 50: 1647-1652 Sayanova (1997) PNAS USA 94: 4211-4216
	Alteration Of Lipoxigenase Enzyme Accumulation And Activity	Porta et al. (1999) Plant and Cell Physiology 40: 850-858.
Protein Composition	- Protein Denaturation - Protein Hydrophilicity	Wisniewski et al.(1999) Physiologia Plantarum 105: 600-608 Steponkus (1998) PNAS USA 95: 14570-14575

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Modulation of Transcription Induced by Low Temperatures	<ul style="list-style-type: none"> <li>- Induced Transcription Factors And Other Dna Binding Proteins</li> <li>- Transcription Of Specific Genes</li> </ul>	<p>Current Protocols in Molecular Biology / edited by Frederick M. Ausubel .. [et al.]. New York : Published by Greene Pub. Associates and Wiley-Interscience : J. Wiley, c1987.</p> <p>Steponkus (1998) PNAS USA 95: 14570-14575</p> <p>Kadyrzhanova et al., Plant Mol Biol (1998) 36(6): 885-895; and</p> <p>Pearce et al., Plant Physiol (1998) 117(3): 787-795</p>
Signal Transduction	Plasma Membrane Proteins	<p>Goodwin et al., Plant Mol Biol (1996) 31(4) 777-781; and</p> <p>Koike et al., Plant Cell Physiol (1997) 38(6): 707-716</p>
Oxygen Scavengers	<ul style="list-style-type: none"> <li>- Glutathione</li> <li>- Accumulation Active O<sub>2</sub> and H<sub>2</sub>O<sub>2</sub> Scavengers</li> </ul>	<p>Kocsy et al., Planta (2000) 210(2): 295-301</p> <p>Tao et al., Cryobiology (1998) 37(1):38-45</p>
Dehydration	<ul style="list-style-type: none"> <li>- Dehydrin</li> <li>- Transcription of mRNA</li> </ul>	<p>Ismail et al., Plant Physiol (1999) 120(1):237-244</p> <p>Kaye et al., Plant Physiol (1998) 116(4): 1367-1377</p>
Metabolism	Soluble Sugars and/or Proline	<p>Wanner et al., (1999) Plant Physiol 120(2): 391-400</p>

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
RNA/DNA Chaperone	Stabilization of RNA/DNA through RNA binding and modulation of RNA translation through RNA binding and or unwinding.	Jiang, Weining et al.,(1997) Journal of Biological Chemistry, 272: 196-202. Fukunaga et al., (1999) Journal of Plant Research, 112: 263-272.
Protein Chaperone	Stabilize protein structure and facilitate protein folding	Forreiter and Nover (1998) Journal of Biosciences 23: 287-302

Other biological activities that can be modulated by the cold responsive genes and their products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table.

Cold responsive genes are characteristically differentially expressed in response to fluctuating cold temperature levels, whether internal or external to an organism or cell. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table reports the changes in transcript levels of various cold responsive genes in the aerial parts of seedlings at 1 and 6 hours at 4°C in the dark as compared to aerial parts of seedlings covered with aluminium foil, and grown at 20°C in the growth chamber.

The data from this time course can be used to identify a number of types of cold responsive genes and gene products, including “early responders” and “delayed responders”. Profiles of these different cold responsive genes are shown in the Table below together with examples of the kinds of associated biological activities.

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	TYPE OF BIOLOGICAL ACTIVITY	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENE PRODUCTS
Upregulated Genes (Level At 1 h $\cong$ 6 h) or (Level At 1 h > 6 h)	Early Responders To Cold	<ul style="list-style-type: none"> <li>- Perception Of Cold</li> <li>- Induction Of Cold Response Signal Transduction Pathways</li> <li>- Initiating Specific Gene Transcription</li> <li>- Osmotic Adjustment</li> <li>- Alteration Of Lipid Composition.</li> <li>- Ice Nucleation Inhibition</li> <li>- Mitigation Of Dehydration By Sequestering Water</li> </ul>	<ul style="list-style-type: none"> <li>- Transcription Factors</li> <li>- Kinases And Phosphatases</li> <li>- Amino Acid Sugar And Metabolite Transporters</li> <li>- Carbohydrate Catabolic And Anabolic Enzymes.</li> <li>- Lipid Biosynthesis Enzymes</li> <li>- Lipid Modification Enzymes, Example Desaturases</li> <li>- Ice Crystal Binding Proteins</li> <li>- Hydrophilic Proteins</li> </ul>

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	TYPE OF BIOLOGICAL ACTIVITY	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENE PRODUCTS
	Stress Response	<ul style="list-style-type: none"> <li>- Repression Of General Biochemical Pathways To Optimize Cold Response Pathways.</li> <li>-Stabilization Of Protein /Enzyme Activity At Low Temperature</li> <li>-Protection Against Oxidative Stress</li> <li>-Anaerobic Metabolism</li> </ul>	<ul style="list-style-type: none"> <li>-Transcription Factors</li> <li>- Kinases And Phosphatases</li> <li>- Protein Stability Factors</li> <li>- mRNA Stability Factors</li> <li>- mRNA Translation Factors</li> <li>- Protein Turnover Factors</li> <li>Oxygen Radical Scavengers, Example- Peroxidases</li> <li>- Energy Generation Enzymes EtOH Detoxification</li> </ul>



GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	TYPE OF BIOLOGICAL ACTIVITY	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENE PRODUCTS
Upregulated Genes (Level At 1h < 6 h)	Delayed Responders To Cold Stress - Cold Acclimation Genes	- Respiration, Photosynthesis And Protein Synthesis - Carbohydrate And Amino Acid Solute Accumulation - Increased Fatty Acid Desaturation To Increase Lipid Membrane Stability - Increased Accumulation Or Activity Of Oxidative Stress Protection Proteins - Stabilization Of Protein /Enzyme Activity At Low Temperature - Protection Against Oxidative Stress - Extracellular Matrix Modification	-Transcription Factors - Kinases And Phosphatases - Protein Stability Factors - mRNA Stability Factors - mRNA Translation Factors - Protein Turnover Factors - Oxygen Radical Scavengers, Peroxidase - Metabolic Enzymes

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	TYPE OF BIOLOGICAL ACTIVITY	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENE PRODUCTS
	Stress Response Genes	<ul style="list-style-type: none"> <li>- Stabilization Of Protein /Enzyme Activity At Low Temperature</li> <li>- Protection Against Oxidative Stress</li> <li>- Anaerobic Metabolism</li> </ul>	<ul style="list-style-type: none"> <li>- Transcription Factors</li> <li>- Kinases And Phosphatases</li> <li>- Protein Stability Factors</li> <li>- mRNA Stability Factors</li> <li>- mRNA Translation Factors</li> <li>- Protein Turnover Factors</li> <li>- Oxygen Radical Scavengers, Example- Peroxidase</li> <li>- Energy Generation Enzymes, Etoh Detoxification</li> </ul>

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	TYPE OF BIOLOGICAL ACTIVITY	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENE PRODUCTS
Downregulated (Level At 1 h $\approx$ 6 h) (Level At 6 h > 1 h)	<ul style="list-style-type: none"> <li>- Early Responder</li> <li>- Repressors Of Cold Stress Metabolism</li> <li>- Genes With Discontinued Expression Or UnsTable mRNA In Cold</li> </ul>	<ul style="list-style-type: none"> <li>- Negative Regulation Of Cold Signal Transduction Pathways Released</li> <li>- Negative Regulation Of Cold Induced Transcription Reduced</li> <li>- Reduction In Gene Expression In Pathways Not Required Under Cold Conditions</li> <li>- Induced mRNA Turnover</li> </ul>	<ul style="list-style-type: none"> <li>- Transcription Factors</li> <li>- Kinases And Phosphatases</li> <li>- Protein Stability Factors</li> <li>- mRNA Stability Factors</li> <li>- mRNA Translation Factors</li> <li>- Protein Turnover Factors</li> <li>- Cold Repressed Metabolic Pathway Proteins</li> <li>- Factors Coordinating And Controlling Central C and N Metabolism</li> <li>- Storage Proteins</li> </ul>

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	TYPE OF BIOLOGICAL ACTIVITY	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENE PRODUCTS
Down-Regulated Transcripts (Level At 1 h > 6 h)	<ul style="list-style-type: none"> <li>- Delayed Responder Repressors Of Cold Stress Metabolism</li> <li>- Genes With Discontinued Expression Or UnsTable mRNA In Cold</li> </ul>	<ul style="list-style-type: none"> <li>- Maintenance Of Cold Induced State Of Metabolism</li> <li>- Reduction In Gene Expression For Pathways Not Required Under Cold Conditions</li> <li>- Induced mRNA Turnover</li> </ul>	<ul style="list-style-type: none"> <li>Transcription Factors</li> <li>- Kinases And Phosphatases</li> <li>- Protein Stability Factors</li> <li>- mRNA Stability Factors</li> <li>- mRNA Translation Factors</li> <li>- Protein Turnover Factors</li> <li>- Cold Repressed Metabolic Pathway Proteins</li> <li>- Factors Coordinating And Controlling Central C and N Metabolism</li> <li>- Storage Proteins</li> </ul>

Further, any desired sequence can be transcribed in similar temporal, tissue, or enviromentally specific patterns as the cold responsive genes when the desired sequence is operably linked to a promoter of a cold responsive gene.

V.B. HEAT RESPONSIVE GENES, GENE COMPONENTS AND PRODUCTS

The ability to endure high temperatures is a major determinant of the geographical distribution and productivity of agricultural crops. Decreases in yield and crop failure frequently occur as a result of aberrant, hot conditions even in areas considered suitable for the cultivation of a given species or cultivar. Only modest increases in the heat tolerance of crop species would have a dramatic impact on agricultural productivity. The development of genotypes with increased heat tolerance would provide a more reliable means to minimize crop losses and diminish the use of energy-costly practices to modify the microclimate.

Changes in temperature in the surrounding environment or in a plant microclimate results in modulation of many genes and gene products. Examples of such heat stress responsive genes and gene products are shown in the Reference, Sequence, Protein Group, Protein Group Matrix, MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff and MA\_clust tables. These genes and/or products are responsible for effects on traits such as plant vigor and seed yield. They were discovered and characterized from a much larger set by experiments designed to find genes whose mRNA products changed in response to high temperatures.

While heat stress responsive polynucleotides and gene products can act alone, combinations of these polynucleotides also affect growth and development. Useful combinations include different heat stress responsive polynucleotides and/or gene products that have similar transcription profiles or similar biological activities, and members of the same or similar biochemical pathways. Whole pathways or segments of pathways are controlled by transcription factor proteins and proteins controlling the activity of signal transduction pathways. Therefore, manipulation of such protein levels is especially useful for altering phenotypes and biochemical activities of plants. In addition, the combination of a heat stress responsive polynucleotide and/or gene product with other environmentally responsive polynucleotide is also useful because of the interactions that exist between stress pathways, pathogen stimulated pathways, hormone regulated pathways, nutritional pathways and development. Here, in addition to polynucleotides having similar transcription profiles and/or biological activities, useful combinations include polynucleotides that may have different transcription profiles, but which participate in common or overlapping pathways. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108576, 108577, 108522, 108523). For transcripts that had higher levels in the samples than the control,

a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Heat genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### Heat Genes Identified By Cluster Analyses Of Differential Expression

##### Heat Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Heat genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108576, 108577, 108522, 108523 of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

##### Heat Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Heat genes. A group in the MA\_clust is considered a Heat pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

##### Heat Genes Identified By Amino Acid Sequence Similarity

Heat genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Heat genes. Groups of Heat genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Heat pathway or network is a group of proteins that also exhibits Heat functions/utilities.

Such heat stress responsive genes and gene products can function either to increase or dampen the above phenotypes or activities either in response to changes in temperature or in the absence of temperature fluctuations.

Further, promoters of heat responsive genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by heat or any of the following phenotypes or biological activities below.

V.B.1. USE OF HEAT STRESS RESPONSIVE GENES TO MODULATE  
PHENOTYPES

Heat stress responsive genes and gene products can be used to alter or modulate one or more of the following phenotypes:

- Heat Tolerance, above 20°C, 23°C, 27°C, 30°C, 33°C, 37°C, 40°C or 42°C
- Of Cells
- Of Organelles
- Of Proteins
- Dehydration Resistance
- Of Cells
- Of Organelles
- Growth Rate
- Whole Plant, including height, bolting time, etc.
- Organs
  - Roots
  - Lateral Roots
  - Leaves
  - Stems
  - Flowers
  - Fruit
- Biomass
- Fresh and Dry Weight during any time in plant life, such as maturation;
- Number, Size, and Weight of
  - Flowers;

- Seeds;
- Branches;
- Leaves;
- Seed Yield
- Number, Size, Weight, Harvest Index
- Fruit Yield
- Number, Size, Weight, Harvest Index
- Stress Responses
  - Mediation of response to desiccation, drought, salt, disease, wounding, cold and other stresses.
- Reproduction

To regulate any of the phenotype(s) above, activity of one or more of the heat stress responsive genes or gene products can be modulated and the plants tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-266) and/or screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and visually inspected for the desired phenotype or metabolically and/or functionally assayed according to Queitsch et al. (2000, The Plant Cell 12: 479-92).

#### V.B.2. USE OF HEAT STRESS RESPONSIVE GENES TO MODULATE BIOCHEMICAL ACTIVITIES

The activities of one or more of the heat stress responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below.

Such biological activities can be measured according to the citations included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATION INCLUDING ASSAY
Cell Growth and Differentiation	-Regulation And Molecular Chaperones	Wisniewski et al. (1999) Physiologia



PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATION INCLUDING ASSAY
	<p>-Maintenance Of Native Conformation (Cytosolic Proteins)</p> <p>-Reactivation Of Aggregation And Protein Folding</p> <p>-Autoregulation Of Heat Shock Response</p> <p>-Regulation Of Translational Efficiency</p> <p>-Regulation Of Kinase Activity</p> <p>-Regulation Of Calcium Mediated Signal Transduction</p>	<p>Plantarum 105: 600-608 Queitsch et al. (2000) The Plant Cell 12: 479-92</p> <p>Lee and Vierling (2000) Plant Physiol. 122: 189- 197 Schwechheimer (1998) Plant Mol Biol 36: 195-204 Shi et al. (1998) Genes and Development 12: 654-66 Wells et al. (1998) Genes and Development 12: 3236-51 Lis et al. (2000) Genes and Development 14: 792-803</p> <p>-Malho, R.(1999) Plant Biology 1: 487-494.</p>

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATION INCLUDING ASSAY
		<p>-Sheen, Jen.(1996) Science 274: 1900-1902.</p> <p>- Farmer, P. et al., (1999.) Biochimica et Biophysica Acta 1434: 6-17.</p>
Gene regulation	<ul style="list-style-type: none"> <li>• Transcriptional Regulation Of Heat Induced Proteins Through DNA Binding Proteins.</li> <li>• Transcriptional Regulation Of Heat Induced Proteins Through Protein-Protein Interactions Between DNA Binding Proteins And Coactivators.</li> </ul>	<p>-Current Protocols in Molecular Biology / edited by Frederick M. Ausubel .. [et al.]. New York : Published by Greene Pub. Associates and Wiley-Interscience : J. Wiley, c1987.</p> <p>-Steponkus (1998) PNAS USA 95: 14570-14575</p> <p>- Gubler et al. (1999) Plant Journal 17: 1-9</p> <p>- Glenn et al. (1999) Journal of Biological Chemistry, 274: 36159-36167</p>

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATION INCLUDING ASSAY
	<ul style="list-style-type: none"> <li>• Transcriptional Regulation Of Heat Induced Proteins Through Protein Phosphorylation And Dephosphorylation</li> <li>• Transcriptional Regulation Of Thermal Stress Induced Genes By Protein-Protein Interactions.</li> <li>• Translational Regulation Of Thermal Stress Induced Messenger Rnas.</li> <li>• Transcriptional Regulation Of Heat Induced Genes Through Chromatin Remodeling.</li> </ul>	<p>- Zhou et al., (1997) EMBO Journal 16:3207-3218.</p> <p>- Sessa et al., (2000) EMBO Journal 19: 2257-2269.</p> <p>- Burnett et al., (2000) Journal of Experimental Botany. 51: 197- 205.</p> <p>- Osterlund et al., (2000) Nature 405: 462-466.</p> <p>- Gross and Watson (1998) Canadian Journal of Microbiology, 44:341-350</p> <p>- Luo, R. X., Dean, D.C. (1999) Journal of the</p>

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATION INCLUDING ASSAY
		National Cancer Institute 91: 1288- 1294.  -Chromatin protocols (1999) edited by Peter B. Becker. Totowa, N.J. : Humana Press.
Cell Structure	<ul style="list-style-type: none"> <li>Thermal Stress Protection By Plasma Membrane Anchored Or Secreted And/Or Cell Wall Associated Proteins.</li> </ul>	<ul style="list-style-type: none"> <li>- Goodwin et al. (1996) Plant Mol Biol 31(4) 777-781; and</li> <li>Koike et al. (1997) Plant Cell Physiol 38(6): 707-716</li> </ul>
Signal Transduction	<ul style="list-style-type: none"> <li>Regulation Of Thermal Stress Pathways And Protein Activity By Protein Kinase And Protein Phosphatase Mediated Phosphorylation And Dephosphorylation Respectively.</li> </ul>	<ul style="list-style-type: none"> <li>- Jonak (1996) Proceedings of the National Academy of Sciences of the United States of America, 93: 11274-11279.</li> <li>- Monroy.et al., (1998) Analytical Biochemistry 265: 183-185.</li> </ul>
Photosynthesis	<ul style="list-style-type: none"> <li>Regulation Of Photoprotection And Repair Of Photosystem II</li> </ul>	<ul style="list-style-type: none"> <li>Schroda et al. (1999) The Plant Cell 11: 1165-178</li> <li>Oh and Lee (1996) J Plant Biol. 39: 301-07</li> </ul>
Stress Response	<ul style="list-style-type: none"> <li>Regulation Of Cytosol</li> </ul>	Dat et al. (1998) Plant

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATION INCLUDING ASSAY
	<p>Peroxide Levels</p> <ul style="list-style-type: none"> <li>Regulation Of Heat Shock Factor Binding</li> <li>Regulation Of Protein Stability During Thermal Stress</li> <li>Nucleocytoplasmic Export Of Heat Shock Protein Mrnas</li> <li>Regulation/Reconfiguration Of Cell Architecture</li> <li>Regulation Of Pathways For Reactivation Of "Damaged" And/Or Denatured Proteins</li> </ul>	<p>Physiol 116: 1351-1357</p> <p>Kurek et al. (1999) Plant Physiol 119: 693-703</p> <p>Storozhenko et al. (1998) Plant Physiol 118: 1005-14</p> <p>Soto et al. (1999) Plant Physiol 120: 521-28</p> <p>Yeh et al. (1997) PNAS 94: 10967-10972</p> <p>Winkler et al. (1998) Plant Physiol 118: 743-50</p> <p>Saavedra et al. (1997) Genes and Development 11: 2845-2856</p> <p>Parsell and Lindquist (1993). Ann. Rev. Genet. 27: 437-496.</p> <p>Parsell and Lindquist (1993). Ann. Rev. Genet. 27: 437-496.</p> <p>Georgopoulos and Welch (1993). Ann Rev. Cell Biol. 9:601-634.</p>

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PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATION INCLUDING ASSAY
	<ul style="list-style-type: none"> <li>• Regulation Of Protein Degradation During Thermal Stress.</li> <li>• Regulation Of Osmotic Potential During Thermal Stress.</li> <li>• Regulation Of Universal Stress Protein Homologue Activity By Phosphorylation And Dephosphorylation.</li> <li>• Regulation Of Dehydrin, LEA-Like And Other Heat Stable Protein Accumulation</li> </ul>	<ul style="list-style-type: none"> <li>- Vierstra, Richard D. (1996) Plant Molecular Biology,32:275-302.</li> <li>- Vierstra, Richard D.; Callis, Judy. (1999) Plant Molecular Biology, 41:435-442.</li> <li>- Liu, J. et al., (1998)Plant Science 134:11-20.</li> <li>- Freestone, P. 1997et al., Journal of Molecular Biology, v. 274: 318-324.</li> <li>- Robertson,A.J. (1994) Plant Physiology 105: 181-190.</li> </ul>

Other biological activities that can be modulated by the heat stress responsive genes and gene products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table.

Heat stress responsive genes are characteristically differentially transcribed in response to fluctuating temperatures, whether internal or external to an organism or cell. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table reports the changes in transcript levels of various heat stress responsive genes in aerial tissues at 1 and 6 hours after plants were placed at 42°C as compared to aerial tissues kept at 20°C growth chamber temperature.

The data from this time course can be used to identify a number of types of heat stress responsive genes and gene products, including "early responders to heat stress," "delayed responders to heat stress," "early responder repressors," and "delayed repressor responders." Profiles of these different heat stress responsive genes are shown in the Table below together with examples of the kinds of associated biological activities.

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITIES / GENE PRODUCTS
Up Regulated Transcripts (Level At 1h ≈ 6h) Or (Level At 1h > 6h)	Early Responders To Heat Stress	<ul style="list-style-type: none"> <li>• Heat Stress Perception</li> <li>• Modulation Of Heat Stress Response Transduction Pathways</li> <li>• Specific Gene Transcription Initiation</li> <li>• Conditional Shift In Preferential Translation Of Transcripts</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> <li>• Transporters</li> <li>• Changes In Cell Membrane Structure</li> <li>• Kinases And Phosphatases</li> <li>• Transcription Activators</li> <li>• Changes In Chromatin Structure And/Or Localized Dna Topology</li> </ul>

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITIES / GENE PRODUCTS
		<ul style="list-style-type: none"> <li>Changes In Cell Architecture To Optimize Cell Adaptation To Heat Stress</li> </ul>	<ul style="list-style-type: none"> <li>Modification Of Pre-Existing Translation Factors By Phosphorylation (Kinases) Or Dephosphorylation (Phosphatases)</li> <li>Synthesis Of New Translation Factors</li> <li>Stability Of Mediators Of Protein-Protein Interaction</li> </ul> <p>Heat Shock Proteins</p> <p>Changes In Organelle Structures, Membranes And Energy-Related Activities</p> <p>Proteins To Catalyse Metabolic Turnover</p>
Up Regulated	"Delayed"		<ul style="list-style-type: none"> <li>Transcription</li> </ul>

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GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITIES / GENE PRODUCTS
Transcripts (Level At 1h < 6h)	Responders  Maintenance Of Heat Stress Response	<ul style="list-style-type: none"> <li>• Maintenance Of Response To Heat Stress</li> <li>• Maintenance Of Protein Stability And Conformation</li> </ul>	<p>Factors</p> <ul style="list-style-type: none"> <li>• Specific Factors (Initiation And Elongation) For Protein Synthesis</li> <li>• Maintenance Of Mrna Stability</li> <li>• Heat Shock Proteins</li> <li>• Changes In Organelle Structures, Membranes And Energy-Related Activities</li> <li>• Proteins To Catalyse Metabolic Turnover.</li> <li>• Stability Of Mediators Of Protein-Protein Interaction</li> </ul>
Down-Regulated Transcripts (Level At 1h ≈ 6h) Or (Level At 6h > 1h)	Early Responder Repressors Of "Normal" State Of Metabolism  Genes With	<ul style="list-style-type: none"> <li>• Negative Regulation Of Heat Stress Response Released</li> <li>• Changes In Biochemical And Signal Transduction</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors And Activators</li> <li>• Change In Protein Structure By Phosphorylation</li> </ul>

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITIES / GENE PRODUCTS
	Discontinued Expression Or Unstable mRNA In Presence Of Heat Stress	Pathways And Processes Operating In Cells • Reorientation Of Metabolism	(Kinases) Or Dephosphorylation (Phosphatases) • Change In Chromatin Structure And/Or Dna Topology
Down-Regulated Transcripts (Level At 1hr > 6hr)	Delayed Repressors Of "Normal" State Of Metabolism  Genes With Discontinued Expression Or Unstable mRNA In Presence Of Heat Stress	• Maintenance Of Heat Stress Response • Maintenance Of Pathways Released From Repression • Changes In Pathways And Processes Operating In Cells • Reorientation Of Metabolism	• Transcription Factors And Activators • Kinases And Phosphatases • Stability Of Factors For Protein Translation

Further, any desired sequence can be transcribed in similar temporal, tissue, or environmentally specific patterns as the heat responsive genes when the desired sequence is operably linked to a promoter of a heat responsive gene.

V.C. DROUGHT RESPONSIVE GENES, GENE COMPONENTS AND PRODUCTS

The ability to endure drought conditions is a major determinant of the geographical distribution and productivity of agricultural crops. Decreases in yield and crop failure frequently occur as a result of aberrant, drought conditions even in areas considered suitable for the cultivation of a given species or cultivar. Only modest increases in the drought tolerance of crop species would have a dramatic impact on agricultural productivity. The development of genotypes with increased drought tolerance would provide a more reliable means to minimize crop losses and diminish the use of energy-costly practices to modify the microclimate.

Drought conditions in the surrounding environment or within a plant, results in modulation of many genes and gene products. Examples of such drought responsive genes and gene products are shown in the Reference and Sequence Tables. These genes and/or products are responsible for effects on traits such as plant vigor and seed yield. They were discovered and characterized from a much larger set by experiments designed to find genes whose mRNA products changed in response to availability of water.

While drought responsive polynucleotides and gene products can act alone, combinations of these polynucleotides also affect growth and development. Useful combinations include different drought responsive polynucleotides and/or gene products that have similar transcription profiles or similar biological activities, and members of the same or similar biochemical pathways. Whole pathways, or segments of pathways are controlled by transcription factor proteins and proteins controlling the activity of signal transduction pathways. Therefore, manipulation of the levels of such proteins is especially useful for altering phenotypes and biochemical activities of plants. In addition, the combination of a drought responsive polynucleotide and/or gene product with another environmentally responsive polynucleotide is also useful because of the interactions that exist between hormone regulated pathways, stress pathways, nutritional pathways and development. Here, in addition to polynucleotides having similar transcription profiles and/or biological activities, useful combinations include polynucleotides that may have different transcription profiles but which participate in a common pathway. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108572, 108573, 108502, 108503, 108504, 108556, 108482, 108483, 108473, 108474, 108477). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript

levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Drought genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or  
5 AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

Drought Genes Identified By Cluster Analyses Of Differential Expression

Drought Genes Identified By Correlation To Genes That Are  
Differentially Expressed

10 As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

15 A pathway or network of Drought genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108572, 108573, 108502, 108503, 108504, 108556, 108482, 108483, 108473, 108474, 108477 of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

20 Drought Genes Identified By Correlation To Genes That Cause  
Physiological Consequences

25 Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Drought genes. A group in the MA\_clust is considered a Drought pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

Drought Genes Identified By Amino Acid Sequence Similarity

30 Drought genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Drought genes. Groups of Drought genes are identified in the Protein Group table. In this table, any protein group that

comprises a peptide ID that corresponds to a cDNA ID member of a Drought pathway or network is a group of proteins that also exhibits Drought functions/utilities.

Such drought responsive genes and gene products can function to either increase or dampen the above phenotypes or activities either in response to drought conditions or in the absence of drought conditions. Further, promoters of drought responsive genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by drought or any of the following phenotypes or biological activities below.

More specifically, drought responsive genes and gene products are useful to or modulate one or more of the following phenotypes:

- Growth
- Roots
- Stems
- Buds
- Leaves
- Development
- Cell Growth
- Leaves
- Fruit Development
- Seed Development
- Senescence
- Stress Responses
- Mediates response to desiccation, drought, salt and cold

Further, any desired sequence can be transcribed in similar temporal, tissue, or environmentally specific patterns as the drought responsive genes when the desired sequence is operably linked to a promoter of a drought responsive gene.

To produce the desired phenotype(s) above, one or more of the drought response genes or gene products can be tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed. As an example, a plant can be transformed according to Bechtold and Pelletier (1998, Methods. Mol. Biol. 82:259-

266) and/or screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and visually inspected for the desired phenotype or metabolically and/or functionally assayed according to Ruzin (1999, In: Plant Microtechnique and Microscopy, Oxford University Press, London) and Khanna-Chopra et al. (1999, BBRC 255:324-7).

5

Alternatively, the activities of one or more of the drought responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities can be measured according to the citations included in the Table below:

GENERAL CATEGORY	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	ASSAY
Cell Growth and Differentiation	Preservation of Leaf Sub-Cellular Structures Including Photosynthetic Apparatus	Jagtap et al. (1998) J Exptl Botany 49:1715-1721
	Preservation of Cell Membrane Structures	Munne-Bosch and Alegre (2000) Planta 210: 925-31
	Regulation of Stomatal development and Physiology	Menke et al. (2000) Plant Physiol. 122:677-686.
	Regulation of Factors Involved in the Drought-adapted change in cell ultrastructure	Harrak et al. (1999) Plant Physiol. 121:557-564.
Physiology	Modulation of Transpiration	Allen et al. (1999) Plant Cell 11: 1785-98 Li et al. (2000) Science 287: 300-303 Burnett et al. (2000) J Exptl Bot 51: 197-205

GENERAL CATEGORY	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	ASSAY
	<p>Modulation of Photosynthesis</p> <p>Regulation of Epicuticular Wax Biosynthesis</p> <p>Regulation of Carotenoid Biosynthesis</p>	<p>Raschke (1987) In: Stomatal function, Zeiger et al., Eds, 253-79</p> <p>Sung and Krieg (1979) Plant Physiol 64: 852-56</p> <p>Rhee et al. (1998) Plant Physiol 116: 901-11</p> <p>Alegre (2000) Planta 210: 925-31</p> <p>Loggini et al (2000) Plant Physiol 119:1091</p>
Stress Response	<p>Modulation of Leaf Rolling to minimize water loss</p> <p>Modulation of Osmolite Synthesis</p> <p>Regulation of gene transcriptional activity specific to</p>	<p>Taiz and Zeiger (1991) In: Plant Physiology, Benjamin/Cummings Publishing Co., Redwood City, pp 346-70</p> <p>Hare et al. (1998) Plant, Cell and Environment 21: 535-553</p> <p>Huan et al. (2000) Plant Physiol 122: 747-756</p> <p>Hare et al. (1999) J. Exptl. Botany 333:413-434.</p>

GENERAL CATEGORY	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	ASSAY
	<p>the establishment of drought tolerance</p> <p>Regulation of protein degradation and reactivation during drought stress condition</p> <p>Modulation/reconfiguration of translation machineries ("recycling" mechanisms) adapTable to drought condition</p>	<p>Lee and Vierling (2000) Plant Physiol. 122: 189-197</p> <p>Lis et al. (2000) Genes and Development 14: 792-803</p>
Signal Transduction	<p>Regulation of Ion Sequestration</p> <p>Regulation of Nuclear Targeted Protein Transport</p> <p>Regulation of Cytoplasmic Ca<sup>+2</sup></p> <p>Regulation of Kinase Synthesis and Activity</p> <p>Modulation of Molecular Chaperone Activity</p>	<p>Bush and Jones (1987) Cell Calcium 8: 455-72</p> <p>Ferringno and Silver (1999) Methods in Cell Biology 58: 107-22</p> <p>Shi et al. (1999) Plant Cell 11: 2393-2406</p> <p>Li et al. (2000) Science 287- 300-03</p> <p>Mayhew et al (1996) Nature 379: 420-26</p>



GENERAL CATEGORY	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	ASSAY
		Kimura et al. (1995) Science 268:1362-1365.

Other biological activities that can be modulated by the drought responsive genes and gene products are listed in the Reference Tables. Assays for detecting such biological activities are described in the Protein Domain table.

Drought responsive genes are characteristically differentially transcribed in response to drought conditions, whether internal or external to an organism or cell. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s) report(s) the changes in transcript levels of various drought responsive genes at 1 and 6 hours after aerial tissues were isolated and left uncovered at room temperature on 3 MM paper, as compared to isolated aerial tissues placed on 3 MM paper wetted with Hoagland's solution.

The data from this time course can be used to identify a number of types of drought responsive genes and gene products, including "early responders," and "delayed responders." Profiles of these different drought responsive genes are shown in the Table below together with examples of the kinds of associated biological activities.

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENE PRODUCTS
Up regulated transcripts (level at 1 hr $\approx$ 6 hr) (level at 1 hr > 6 hr)	Early responders to drought	Drought perception leading to the establishment of tolerance to drought  Modulation of drought response transduction	Transcription factors Transporters  Change in cell membrane structure

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENE PRODUCTS
		<p>pathways</p> <p>Specific gene transcription initiation</p> <p>Conditional shift in preferential translation of transcripts</p> <p>Changes in cell architecture to optimize cell adaptation to heat stress</p> <p>Changes in cell division cycle</p>	<p>Kinases and phosphatases</p> <p>Transcription activators Change in chromatin structure and/or localized DNA topology</p> <p>Modification of pre- existing translation factors by phosphorylation (kinases) or dephosphorylation (phosphatases) Synthesis of new translation factors</p> <p>Stability of mediators of protein-protein interaction</p> <p>Synthesis and/or stability of factors regulating cell division</p>
Up regulated transcripts (level at 1 hr < 6 hr)	Maintenance of drought response	Maintenance of response to drought and maintenance of	Transcription factors Specific factors (initiation and elongation) for protein

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENE PRODUCTS
	"Delayed" responders	<p>drought-tolerance mechanisms</p> <p>Maintenance of mechanisms effective for ions sequestration, osmolite biosynthesis, nuclear protein transport, regulation of cytoplasmic Ca<sup>2+</sup>, and regulation of proteins effective for maintaining protein stability and conformation</p> <p>Maintenance of cellular structures</p>	<p>synthesis</p> <p>RNA-binding proteins effective for mRNA stability</p> <p>Change in chromatin structure and/or DNA topology</p> <p>Stability of mediators of protein-protein interaction</p> <p>Stability of factors to effectively utilize pre-existing translation machinery ("recycling" mechanisms) under drought condition</p> <p>Stability of mediators of protein-protein interaction</p>
Down-regulated transcripts	Early responder repressors of "normal"	Negative regulation of drought response	Transcription factors and activators

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITIES OF GENE PRODUCTS
(level at 1 hr $\approx$ 6 hr) (level at 6 hr > 1 hr)	state of metabolism  Genes with discontinued expression or unstable mRNA in presence of water stress	inducible pathways released Changes in biochemical and signal transduction pathways and processes operating in cells	Change in protein structure by phosphorylation (kinases) or dephosphorylation (phosphatases) Change in chromatin structure and/or DNA topology
Down-regulated transcripts (level at 1 hr > 6 hr)	Delayed repressors of "normal" state of metabolism  Genes with discontinued expression or unstable mRNA in presence of water stress	Maintenance of drought response Maintenance of pathways released from repression Changes in pathways and processes operating in cells	Transcription factors and activators Kinases and phosphatases Stability of factors for protein translation

#### Use of Promoters of Drought Responsive Genes

Promoters of Drought responsive genes are useful for transcription of any desired polynucleotide or plant or non-plant origin. Further, any desired sequence can be transcribed in a similar temporal, tissue, or environmentally specific patterns as the Drought responsive genes where the desired sequence is operably linked to a promoter of a Drought responsive gene. The protein product of such a polynucleotide is usually synthesized in the same cells, in response to the same stimuli as the protein product of the gene from which the promoter was derived. Such

promoter are also useful to produce antisense mRNAs to down-regulate the product of proteins, or to produce sense mRNAs to down-regulate mRNAs via sense suppression.

V.D. WOUNDING RESPONSIVE GENES, GENE COMPONENTS AND  
PRODUCTS

Plants are continuously subjected to various forms of wounding from physical attacks including the damage created by pathogens and pests, wind, and contact with other objects.

5 Therefore, survival and agricultural yields depend on constraining the damage created by the wounding process and inducing defense mechanisms against future damage.

Plants have evolved complex systems to minimize and/or repair local damage and to minimize subsequent attacks by pathogens or pests or their effects. These involve stimulation of cell division and cell elongation to repair tissues, induction of programmed cell death to isolate  
10 the damage caused mechanically and by invading pests and pathogens, and induction of long-range signaling systems to induce protecting molecules, in case of future attack. The genetic and biochemical systems associated with responses to wounding are connected with those associated with other stresses such as pathogen attack and drought.

Wounding results in the modulation of activities of specific genes and, in consequence, of  
15 the levels of key proteins and metabolites. These genes, called here wounding responsive genes, are important for minimizing the damage induced by wounding from pests, pathogens and other objects. Examples of such wounding responsive genes, gene components and products are shown in the Reference, Sequence, Protein Group, Protein Group Matrix, MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff, and MA\_clust tables. They can be  
20 active in all parts of a plant and so where, when and to what extent they are active is crucial for agricultural performance and for the quality, visual and otherwise, of harvested products. They were discovered and characterized from a much larger set of genes by experiments designed to find genes whose products changed in response to wounding.

Manipulation of one or more wounding responsive gene activities are useful to modulate  
25 the biological activities and/or phenotypes listed below. Wounding responsive genes and gene products can act alone or in combination with genes induced in other ways. Useful combinations include wounding responsive genes and/or gene products with similar transcription profiles, similar biological activities, or members of functionally related biochemical pathways. Whole pathways or segments of pathways are controlled by transcription factor proteins and proteins  
30 controlling the activity of signal transduction pathways. Therefore, manipulation of the levels of such proteins is especially useful for altering phenotypes and biochemical activities of plants.

The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108574, 108575, 108524, 108525, and Wounding (relating to SMD 3714, SMD 3715) ). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

Wounding genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

#### Wounding Genes Identified By Cluster Analyses Of Differential Expression

##### Wounding Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of Wounding genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108574, 108575, 108524, 108525, and Wounding (relating to SMD 3714, SMD 3715) of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

##### Wounding Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Wounding genes. A group in the MA\_clust is considered a Wounding pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

Wounding Genes Identified By Amino Acid Sequence Similarity

Wounding genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Wounding genes.

Groups of Wounding genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Wounding pathway or network is a group of proteins that also exhibits Wounding functions/utilities.

Such wounding responsive genes and gene products can function either to increase or dampen the phenotypes and activities below, either in response to wounding or in the absence of wounding.

Further, promoters of wounding responsive genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by wounding or any of the following phenotypes or biological activities below.

V.D.1. USE OF WOUNDING-RESPONSIVE GENES TO MODULATE  
PHENOTYPES

Wounding responsive genes and gene products can be used to alter or modulate one or more of the following phenotypes:

- Growth Rate
- Whole Plant
  - Height
  - Width
  - Flowering Time
- Organs
  - Coleoptile Elongation
  - Young Leaves
  - Roots
  - Lateral Roots
  - Tuber Formation
  - Flowers
  - Fruit



- Seeds
- Biomass
- Fresh And Dry Weight During Any Time In Plant Life, Such As At Maturation
- Number Of Flowers
- Number Of Seeds
- Seed Yield
- Number
- Size
- Weight
- Harvest Index
- Content and Composition, e.g., Amino Acid, Nitrogen, Oil, Protein, And Carbohydrate
- Fruit Yield
- Number
- Size
- Weight
- Harvest Index
- Post Harvest Quality
- Content And Composition, e.g., Amino Acid, Carotenoid, Jasmonate, Protein, And Starch
- Seed and Fruit Development
- Germination Of Dormant And Non-Dormant Seeds
- Seed Viability
- Seed Reserve Mobilization
- Fruit Ripening
- Initiation Of The Reproductive Cycle From A Vegetative State
- Flower Development Time
- Insect Attraction For Fertilization
- Time To Fruit Maturity
- Senescence
- Fruits, Fruit Drop

- Leaves
- Stress And Disease Responses
- Drought
- Heat And Cold
- 5    - Wounding By Any Source, Including Wind, Objects, Pests And Pathogens
- Uv And High Light Damage
  - Insect, Fungus, Virus, Worm, Nematode Damage

To regulate any of the phenotype(s) above, activities of one or more of the wounding responsive genes or gene products can be modulated and the plants can be tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and assayed, for example, in accordance with Johnson et.al. (1998) Plant Physiol 116:643-649, Reymond et.al. (2000) Plant Cell 12 707-720, or Keith et.al. (1991) Proc. Nat. Acad. Sci.USA 888821 8825.

V.D.2. USE OF WOUNDING-RESPONSIVE GENES TO MODULATE  
BIOCHEMICAL ACTIVITIES

5      The activities of one or more of the wounding responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities are documented and can be measured according to the citations included in the Table below:

PROCESS	BIOLOGICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Plant Tissue Proliferation	Cell Damage Repair; Cell Division	Flanders (1990) J. Cell Biol. 110: 1111-1122
Wound Induced Pathways Providing Defense Against Pests And Pathogens	Synthesis Of Jasmonic And Salicylic Acids And The Pathways Induced By These Signaling Molecules. Induction Of Jasmonic Acid Independent Defense Pathways. Induction Of Lipxygenase, Thionins And Nodulins	Reymond, P and Farmer E.E. Current Opinion in Plant Biology 1998 1:404-411 Creelman, RA and Mullet, J.E. (1997) Ann Rev. Plant Physiol Mol Biol 48: 355-387 Leon et al. 1998 Mol Gen Genet 254: 412-419 Titarentko et al. 1997 Plant Physiol 115: 817-826
	Cell Wall Degradation, Ethylene Formation, Systemic Signaling And Induction Of Defense Related Genes	Rajo, E. et al. 1998. Plant J 13:153-165 Ryan, CA and Pearce, G. 1998. Ann Rev. Cell Dev. Biol 14: 1-17
	Specific Rnase Induction	Reymond, P. et al. 2000 . Plant Cell 12:707-720 Glazebrook, J. 1999. Current Opinion in Plant Biol. 2: 280-

PROCESS	BIOLOGICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
		286 O'Donnel P. J., et al. 1996 Science 274: 1914-1917 Rojo et al. 1999. Plant J. 20: 135-142 Merkouropoulos G. et al. 1999 Planta 208: 212-219 Kariu et al. 1998. Bioscience Biotechnology and Biochemistry 62: 1144-1151 Mcoann et al. 1997 PNAS 94: 5473-5477
Other Stress Induced Pathways	Absciscic Acid Formation And Its Signaling Pathway Cold Responsive Genes and Pathways Drought Induced Dehydrins And Pathways	Carrera, E and Prat, S. 1998. Plant J 15: 767-771 Chao et. al. 1999. Plant Physiol 120: 979-992
Modified Lipid Metabolism          Modified Sugar And Energy Metabolism	Membrane Lipid Synthesis Including Omega-3 Fatty Acid Desaturase Lipases Lipid Transfer Proteins  Induction Of Glycohydrolases And Glycotransferases, Amylases Induction Of	Martin, M et al. 1999 Europe J. Biochem 262: 283-290

PROCESS	BIOLOGICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Modified Protein And Nitrogen Metabolism	Aminotransferases, Arginase, P roteases And Vegetative Storage Proteins, Aromatic Amino Acid Synthesis	
Secondary Metabolite Induction	Aromatic Amino Acid Synthesis And Secondary Metabolites	Keith, B et al. 1991 PNAS 88: 8821-8825

Other biological activities that can be modulated by wound responsive genes and their products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table.

The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table reports the changes in transcript levels of various wound responsive genes in the aerial parts of a plant, 1 and 6 hours after the plants were wounded with forceps. The comparison was made with aerial tissues from unwounded plants.

The data from this time course reveal a number of types of wound responsive genes and gene products, including "early responders," and "delayed responders." Profiles of the individual wounding responsive genes are shown in the Table below together with examples of the kinds of associated biological activities that are modulated when the activities of one or more such genes vary in plants.

TRANSCRIPT LEVELS	TYPES OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
Up Regulated Transcripts (Level At 1h $\approx$ 6h) Or (Level At 1h > 6h)	Early Responders To Wounding	<ul style="list-style-type: none"> <li>• Induction Of Key Signaling Pathways Within And Between Cells</li> <li>• Modulation Of Wounding And Stress Induced Signal Transduction Pathways</li> <li>• Specific Gene Transcription Initiation</li> <li>• Induction Of Repair Processes Or Cell Death</li> <li>• Reorientation Of Metabolism, Including Management Of Active Oxygen</li> <li>• Movement Of Wound</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> <li>• Kinases And Phosphatases</li> <li>• Jasmonic Acid, Salicylic Acid And Nitric Oxide Pathway Proteins.</li> <li>• Glycohydrolases</li> <li>• Dehydrins</li> <li>• Rnases</li> <li>• Metabolic Enzymes</li> <li>• Nodulins</li> <li>• Cell Division And Cell Wall Proteins</li> <li>• Cold Response Proteins</li> <li>• Lipoxigenase</li> <li>• Jacalin</li> <li>• Proteins To Detoxify Active Oxygen Species</li> <li>• Systemin</li> </ul>

TRANSCRIPT LEVELS	TYPES OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
		<p>Induced Signals Through Plant</p> <ul style="list-style-type: none"> <li>• Synthesis Of Phytoalexins And Secondary Metabolites</li> </ul>	<ul style="list-style-type: none"> <li>• Biosynthetic Enzymes</li> </ul>
Up Related Transcripts ( Level At 1h < 6h)	<p>Delayed Responders</p> <p>Genes Involved In Wounding Response At Distant Sites From Wound.</p> <p>Genes Involved In Maintenance Of Wounding Response</p>	<ul style="list-style-type: none"> <li>• Maintenance Of Defence Pathways</li> <li>• Maintenance Of Reorientated Metabolism</li> <li>• Maintenance Of Wound Response</li> <li>• Programmed Cell Death In Selected Cells</li> <li>• Reorientation Of Metabolism</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> <li>• Kinases And Phosphatases</li> <li>• Jasmonic Acid, Salicylic Acid And Nitric Oxide Pathway Proteins</li> <li>• Glycohydrolases</li> <li>• Dehydrins</li> <li>• Rnases</li> <li>• Metabolic Enzymes</li> <li>• Nodulins</li> <li>• Cold Response Proteins</li> <li>• Lipoxygenase</li> </ul>

TRANSCRIPT LEVELS	TYPES OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
		<ul style="list-style-type: none"> <li>• Movement Of Wound Induced Signals Through Plant</li> <li>• Synthesis Of Phytoalexins And Secondary Metabolites</li> </ul>	<ul style="list-style-type: none"> <li>• Jacalin</li> <li>• Proteins To Detoxify Active Oxygen Species</li> <li>• Cell Division And Cell Wall Proteins</li> <li>• Systemin</li> <li>• Biosynthetic Enzymes</li> </ul>
Down – Regulated Transcripts (Level At 1h $\approx$ 6h) Or (Level At 6 Hr > 1h)	<ul style="list-style-type: none"> <li>• Early Responder Repressors Of Wounding Response State</li> <li>• Genes With Discontinued Expression Or UnsTable</li> </ul>	<ul style="list-style-type: none"> <li>• Negative Regulation Of Wounding Response Pathways Released</li> <li>• Changes In Pathways And Processes Operating In Cells</li> </ul>	<ul style="list-style-type: none"> <li>• Transcription Factors</li> <li>• Change In Protein Structure By Phosphory-Laton (Kinases) Or Dephos-Phorylation (Phosphatases)</li> <li>• Change In Chromatin Structure And Or Dna Topology</li> <li>• Local Changes In Regulatory Proteins, Metabolic Enzymes, Transporters Etc.</li> </ul>



TRANSCRIPT LEVELS	TYPES OF GENES	PHYSIOLOGICAL CONSEQUENCES	EXAMPLES OF BIOCHEMICAL ACTIVITY
	mRNA Following Wounding		
Down – Regulated Transcripts (Level At 1hr > 6h)	Delayed Repressors Of Wounding Response State          Genes With Discontinued Expression Or Unstable mRNA Following Wounding	<ul style="list-style-type: none"> <li>Negative Regulation Of Wounding Response Pathways Released</li> <li>Change In Pathways And Process Operating In Cells</li> <li>Programmed Cell Death</li> </ul>	<ul style="list-style-type: none"> <li>Transcription Factors, Phosphatases, Kinases</li> <li>Changes In Protein Complex Structures</li> <li>Chromatin Restructuring Proteins</li> <li>Local Changes In Regulatory Proteins, Metabolic Enzymes, Transporters Etc.</li> <li>Most Proteins In Selected Cells Undergoing Death</li> </ul>

Further, any desired sequence can be transcribed in similar temporal, tissue, or environmentally specific patterns as the wounding responsive genes when the desired sequence is operably linked to a promoter of a wounding responsive gene.

V.E. METHYL JASMONATE (JASMONATE) RESPONSIVE GENES, GENE  
COMPONENTS AND PRODUCTS

Jasmonic acid and its derivatives, collectively referred to as jasmonates, are naturally occurring derivatives of plant lipids. These substances are synthesized from linolenic acid in a lipoxxygenase-dependent biosynthetic pathway. Jasmonates are signalling molecules which have been shown to be growth regulators as well as regulators of defense and stress responses. As such, jasmonates represent a separate class of plant hormones.

Changes in external or internal jasmonate concentration result in modulation of the activities of many genes and gene products. Examples of such "jasmonate responsive" genes and gene products are shown in the Reference and Sequence Tables. These genes and/or products are responsible for effects on traits such as plant vigor and seed yield, especially when plants are growing in the presence of biotic or abiotic stresses. They were discovered and characterized from a much larger set of genes by experiments designed to find genes whose mRNA products changed in concentration in response to application of methyl jasmonate to plants.

Manipulation of one or more jasmonate responsive gene activities are useful to modulate the biological activities and/or phenotypes tested below. Jasmonate response genes and gene products can act alone or in combination. Useful combinations include jasmonate responsive genes and/or gene products with similar transcription profiles, similar biological activities, or members of the same co-regulated or functionally related biochemical pathways. Whole pathways or segments of pathways are controlled by transcription factor proteins and proteins controlling the activity of signal transduction pathways. Therefore, manipulation of such protein levels is especially useful for altering phenotypes and biochemical activities

Such jasmonate responsive genes and gene products can function to either increase or dampen the phenotypes or activities below either in response to changes in jasmonate concentration or in the absence of jasmonate fluctuations. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the experiment (see EXPT ID: 108568, 108569, 108555). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

MeJA genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

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MeJA Genes Identified By Cluster Analyses Of Differential Expression

MeJA Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

A pathway or network of MeJA genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108568, 108569, 108555 of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

MeJA Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of MeJA genes. A group in the MA\_clust is considered a MeJA pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

MeJA Genes Identified By Amino Acid Sequence Similarity

MeJA genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis MeJA genes. Groups of MeJA genes are identified in the Protein Group table. In this table, any protein group that comprises a

peptide ID that corresponds to a cDNA ID member of a MeJA pathway or network is a group of proteins that also exhibits MeJA functions/utilities.

Further, promoters of jasmonate responsive genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by jasmonate or any of the  
5 following phenotypes or biological activities below.

V.E.1. USE OF JASMONATE RESPONSIVE GENES TO MODULATE  
PHENOTYPES:

Jasmonate responsive genes and their gene products can be used to alter or modulate one or more of the following phenotypes:

- Growth rate

Whole Plant, including Height, Flowering Time, etc.

Seedling

Organ

Coleoptile Elongation

Young Leaves

Roots

Lateral Roots

Tuber Formation

Flowers

Fruit

Seeds

Biomass

Fresh and Dry Weight during any time in plant life, including maturation and senescence

Number of Flowers

Number of Seeds

- Secondary Metabolite Accumulation

Alkaloids

Anthocyanins

Paclitaxel and Related Taxanes

Rosmarinic

Seed Yield

- Number, Size, Weight, Harvest Index

- Content and Composition, e.g., Amino Acid, Jasmonate, Oil, Protein, and

Starch

Fruit Yield

- Number, Size, Weight, Harvest Index, Post Harvest Quality
- Content and Composition e.g., Amino Acid, Carotenoid, Jasmonate, Protein, Starch
- Seed and Fruit Development
- Germination of Dormant and Non-Dormant Seeds
- Seed Viability
- Seed Reserve Mobilization
- Fruit Ripening
- Initiation of the Reproductive cycle from a vegetative state
- Flower Development Time
- Insect Attraction for Fertilization
- Time to Fruit Maturity
- Senescence
- Fruits, Fruit Drop
- Leaves
- Stress and Disease Responses
- Drought
- Wounding
- UV damage
- Insect, Fungus, Virus, Worm damage

Further, any desired sequence can be transcribed in similar temporal, tissue, or environmentally specific patterns as the jasmonate responsive genes when the desired sequence is operably linked to a promoter of a jasmonate responsive gene.

To improve any of the phenotype(s) above, activities of one or more of the jasmonate responsive genes or gene products can be modulated and the plants can be tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be assayed, for example, in accordance to citations described below.

#### V.E.2. USE OF JASMONATE-RESPONSIVE GENES TO MODULATE BIOCHEMICAL ACTIVITIES:

The activities of one or more of the jasmonate responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities are documented and can be measured according to the citations included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Turnover of proteins	- Induction of various proteases, ubiquitin and proteosome components and turnover of RNA polymerases and translation initiation factors - Reduction in many ribosomal proteins	This study. Standard biochemical assays.
Activation of nitrogen metabolism	Induction of glutamine synthetase, many aminotransferases, vegetative storage proteins	Crawford (1995) Plant Cell 7, 859-868 This study. Standard biochemical assays.
Lipid turnover	Induction of various lipases, desaturases, and reduction of lipid transfer protein mRNAs	This study. Standard biochemical assays.
Sugar metabolism	Induction of sugar transporters, UDP glucosyltransferases, other transferases	This study. Standard biochemical assays.



Glycolysis and central carbon metabolism	Induction of glycolytic related enzymes . Example, glucose 6-phosphate dehydrogenase, glyceraldehyde-3-phosphate dehydrogenase, phosphoglycerate kinase, phosphoglucomutase ATP synthase	This study. Standard biochemical assays.
Chlorosis	Degradation of Chlorophyll	Tsuchiya et al. (1999) Proc. Natl. Acad. Sci. U SA 96:15362-15367
	Inhibition of Photosynthesis Related Proteins	Reinbothe et al. (1993) J. Biol. Chem. 268, 10606-10611
Carbon Assimilation and turnover	Induction of chlorophyll ab binding protein precursor	Reinbothe et al. (1993) J. Biol. Chem. 268, 10606-10611
Jasmonate metabolism	Induction of lipid biosynthesis, myrosinase and jacalin	This study. Standard biochemical assays.
Jasmonate mediated signal transduction	Receptor binding	Cho and Pai (2000) Mol Cells 10, 317-324
	Protein kinases	Lee et al. (1998) Mol. Gen. Genet. 259, 516-522  Seo et al. (1999) Plant Cell 11, 289-298  Yoon et al. (1999) Plant Mol. Biol. 39, 991-1001

	Ubiquitination of Repressor Proteins	Xie et al. (1998) Science 280, 1091-1094
	Calcium Flux regulators	Bergey and Ryan (1999) Plant Mol. Biol. 40, 815-823
	Transcription Activators. Example- induction of various zinc finger, myb and AP-2 related factors	Xiang et al. (1996) Plant Mol. Biol. 32, 415-426  Menke et al. (1999) EMBO J. 18, 4455-4463
Response to Cell Membrane Damage	Lipid Peroxidation	Dubery et al. (2000) Mol. Cell Biol. Res. Commun. 3, 105-110
Cell Elongation	Inhibition of incorporation of Glucose into Cell Wall Saccharides	Burnett et al. (1993) Plant Physiol. 103, 41-48
Cell Organization and Division	- Reductions in tropomyosin related proteins and certain cyclins - Induction of actins and tubulins	Ishikawa et al. (1994) Plant Mol. Biol. 26, 403-414

Cell Wall Turnover and modulation	<ul style="list-style-type: none"> <li>- Induction of cell wall proteins, glycine-rich proteins, annexins, pectate lyase and pectin esterases</li> <li>- Reductions in various dehydrins and expansins</li> </ul>	<p>Creelman et al. (1992) Proc. Natl. Acad. Sci. USA 89, 4938-4941</p> <p>Garcia-Muniz et al. (1998) Plant Mol. Biol. 38, 623-632</p> <p>Norman et al (1999) Mol. Plant Microbe Interact. 12, 640-644</p>
Stress, Disease, and Pathogen Resistance	<p>Induction of antifungal proteins, wounding responsive proteins, dehydrins, heat shock type proteins and elicitor response proteins</p>	<p>Hildmann et al. (1992) Plant Cell 4, 1157-1170</p> <p>Reinbothe et al. (1994) Proc. Natl. Acad. Sci. USA 91, 7012-7016</p> <p>Moons et al. (1997) Plant Cell 9, 2243-2259</p> <p>Richard et al. (2000) Plant Mol. Biol. 43, 1-10</p> <p>Van Wees et al. (2000) Proc. Natl. Acad. Sci. USA 97, 8711-8716</p>

	Phytoalexin Biosynthesis	Creelman et al. (1992) Proc. Natl. Acad. Sci. USA 89, 4938-4941  Choi et al. (1994) Proc. Natl. Acad. Sci. USA 91, 2329-2333
	Biosynthesis of phenolics	Doares et al., (1995) Proc. Natl. Acad. Sci. USA 92, 4095-5098
	Production of Protease Inhibitors	Botella et al. (1996) Plant Physiol 112, 1201-1210
	Defense Gene Transcription in Response to UV	Mason et al. (1993) Plant Cell 5, 241-251  Schaller et al. (2000) Planta 210, 979-984
Secondary Metabolite biosynthesis	Fruit Carotenoid Composition	Czapski and Saniewski (1992) J. Plant Physiol. 139, 265-268
	Palitaxel and Related Taxanes	Yukimune et al. (1996) Nature Biotech. 14, 1129-1132

	Alkaloids	Aerts et al. (1994) Plant J. 4, 635-643 Geerlings et al. (2000) J. Biol. Chem. 275, 3051-3056
	Anthocyanins	Franceschi et al. (1991) Proc. Natl. Acad. Sci. USA 83, 6745-6749
	Rosmarinic	Mizukami et al., (1993) Plant Cell Reprod. 12, 706-709
	Activation of Ethylene- forming Enzyme and Production of Ethylene	Czapski and Saniewski (1992) J. Plant Physiol. 139, 265-268

Other biological activities that can be modulated by the jasmonate responsive genes and their products are listed in the Reference Tables. Assays for detecting such biological activities are described in the Domain section of the Reference Tables.

Jasmonate responsive genes are characteristically differentially transcribed in response to fluctuating jasmonate levels or concentrations, whether internal or external to an organism or cell. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s) report(s) the changes in transcript levels of various jasmonate responsive genes in the aerial parts of a seedling at 1 and 6 hours after being sprayed with Silwet L-77 solution enriched with methyl jasmonate as compared to seedlings sprayed with Silwet L-77 alone.

The data from this time course reveal a number of types of jasmonate responsive genes and gene products, including "early responders" and "delayed responders". Profiles of the individual kinds of jasmonate responsive genes are shown in the Table below, together with examples of the kinds of associated biological activities that are modulated when the activities of such genes vary.

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	TYPE OF BIOLOGICAL ACTIVITY	EXAMPLES OF BIOCHEMICAL ACTIVITY
Upregulated genes (Level at 1 hour $\geq$ 6 hours). (Level at 1 hour > 6 hours)	Early Responders to Jasmonate	Binding and Perception of Jasmonate  Transduction of Jasmonate signal transduction response pathways  Initiation of Specific Gene Transcription to reorientate metabolism	Transcription Factors Transporters  Kinases, Phosphatases, Leucine-rich Repeat Proteins (LRRs), GTP- binding proteins (G- proteins), calcium- binding proteins and calcium responsive proteins  Proteases, lipases, glutamine synthetase (GS), arginase, aminotransferases, glycosyltransferases, sugar transporters, cell wall proteins, methyl transferases, glycolytic enzymes.
Upregulated genes (Level at 1 hour < 6 hours)	Delayed Jasmonate Responders	Maintenance of Metabolism under high Jasmonate	Enzymes of methyl jasmonate-induced pathways, including dehydrin, phytoalexin, phenolic, carotenoid, alkaloid and anthocyanin

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	TYPE OF BIOLOGICAL ACTIVITY	EXAMPLES OF BIOCHEMICAL ACTIVITY
		Jasmonate signal Tranduction Response Pathways  Gene Transcription to Reorientate Metabolism          Gene Transcription to Maintain Reorientated Metabolism	biosynthesis.  Transcription factors, Transporters, Kinases and phosphatases  Proteases, Lipases, Glutaminae Synthetase, Arginase, Aminotransferases, Lipid Peroxidases, Glycosyltransferases, Sugar transporters, Cell Wall Proteins, Glycolytic Enzymes, Chlorophyll Binding Proteins  Transcription factors, kinases, phosphatases, LRRs, G-proteins

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	TYPE OF BIOLOGICAL ACTIVITY	EXAMPLES OF BIOCHEMICAL ACTIVITY
		Reorient Cell Division and Cell Development	Actins, Tubulins, Myosins Cyclins, Cyclin-dependent Kinases (CDPKs)  Glycosyl Transferases, Glycosyl hydrolases, Expansins, Extensins, O- Methyl Transferases  Arabinogalactan- proteins (AGPs), Enzymes of Lipid Biosynthesis, Cutinase
Down regulated transcripts (level at 1 hour = 6 hours) ( level at 6 hours >1 hour)	Early responders of Jasmonate  Genes with discontinued expression or unsTable mRNA following Jasmonate uptake	Release of Suppression of Jasmonate Induced Pathways  Reorientation of metabolism	Transcription Factors, Kinases, Phosphatases, LRRs, G-Proteins, Chromatin Restructuring proteins,  Ribosomal proteins, Translation Factors, Histones, RNA polymerases, Pectin esterase, Lipid transfer proteins



GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	TYPE OF BIOLOGICAL ACTIVITY	EXAMPLES OF BIOCHEMICAL ACTIVITY
Down regulated transcripts (level at 1 hour > 6 hours)	Genes with Discontinued expression or UnsTable mRNA Following Jasmonate uptake	Negative Regulation of Jasmonate Induced Pathways Released.  Reorientation of metabolism	Transcription factors Kinases, Phosphatases Chromatin Restructuring Proteins, LRRs, G-proteins  Ribosomal proteins, Translation Factors, Histones RNA Polymerases, Cyclins Pectin esterase, Lipid Transfer Proteins

#### Use of Promoters of Jasmonate Responsive Genes

Promoters of Jasmonate responsive genes are useful for transcription of any desired polynucleotide or plant or non-plant origin. Further, any desired sequence can be transcribed in a similar temporal, tissue, or environmentally specific patterns as the Jasmonate responsive genes where the desired sequence is operably linked to a promoter of a Jasmonate responsive gene. The protein product of such a polynucleotide is usually synthesized in the same cells, in response to the same stimuli as the protein product of the gene from which the promoter was derived. Such promoter are also useful to produce antisense mRNAs to down-regulate the product of proteins, or to produce sense mRNAs to down-regulate mRNAs via sense suppression.

V.F. REACTIVE OXYGEN RESPONSIVE GENES, GENE COMPONENTS AND  
H<sub>2</sub>O<sub>2</sub> PRODUCTS

Often growth and yield are limited by the ability of a plant to tolerate stress conditions, including pathogen attack, wounding, extreme temperatures, and various other factors. To combat such conditions, plant cells deploy a battery of inducible defense responses, including triggering an oxidative burst. The burst of reactive oxygen intermediates occurs in time, place and strength to suggest it plays a key role in either pathogen elimination and/or subsequent signaling of downstream defense functions. For example, H<sub>2</sub>O<sub>2</sub> can play a key role in the pathogen resistance response, including initiating the hypersensitive response (HR). HR is correlated with the onset of systemic acquired resistance (SAR) to secondary infection in distal tissues and organs.

Changes in reactive oxygen, such as H<sub>2</sub>O<sub>2</sub> or O<sub>2</sub><sup>-</sup>, in the surrounding environment or in contact with a plant results in modulation of the activities of many genes and hence levels of gene products. Examples of such reactive oxygen responsive genes and gene products are shown in the Reference, Sequence, Protein Group, Protein Group Matrix, MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff and MA\_clust tables. These genes and/or products are responsible for effects on traits such as plant vigor and seed yield. The genes were discovered and characterized from a much larger set by experiments designed to find genes whose mRNA products changed in response to application of reactive oxygen, such as H<sub>2</sub>O<sub>2</sub>, to plants.

Manipulation of one or more reactive oxygen responsive gene activities are useful to modulate the following biological activities and/or phenotypes listed below. Reactive oxygen responsive genes and gene products can act alone or in combination. Useful combinations include reactive oxygen responsive genes and/or gene products with similar transcription profiles, similar biological activities, or members of the same or functionally related biochemical pathways. Whole pathways or segments of pathways are controlled by transcription factor proteins and proteins controlling the activity of signal transduction pathways. Therefore, manipulation of such protein levels is especially useful for altering phenotypes and biochemical activities of plants.

Such reactive oxygen responsive genes and gene products can function to either increase or dampen the above phenotypes or activities either in response to changes in reactive oxygen concentration or in the absence of reactive oxygen fluctuations. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff Table(s) reports the transcript levels of the

experiment (see EXPT ID: 108582, 108583, 108537, 108538, 108558, and H2O2 (relating to SMD 7523)). For transcripts that had higher levels in the samples than the control, a "+" is shown. A "-" is shown for when transcript levels were reduced in root tips as compared to the control. For more experimental detail see the Example section below.

5        Reactive Oxygen genes are those sequences that showed differential expression as compared to controls, namely those sequences identified in the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff tables with a "+" or "-" indication

10        Reactive Oxygen Genes Identified By Cluster Analyses Of Differential Expression

15        Reactive Oxygen Genes Identified By Correlation To Genes That Are Differentially Expressed

As described above, the transcription profiles of genes that act together are well correlated. Applicants not only have identified the genes that are differentially expressed in the microarray experiments, but also have identified the genes that act in concert with them. The MA\_clust table indicates groups of genes that have well correlated transcription profiles and therefore participate in the same pathway or network.

20        A pathway or network of Reactive Oxygen genes is any group in the MA\_clust that comprises a cDNA ID that also appears in Expt ID 108582, 108583, 108537, 108538, 108558, and H2O2 (relating to SMD 7523) of the MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table(s).

25        Reactive Oxygen Genes Identified By Correlation To Genes That Cause Physiological Consequences

Additionally, the differential expression data and the phenotypic observations can be merged to identify pathways or networks of Reactive Oxygen genes. A group in the MA\_clust is considered a Reactive Oxygen pathway or network if the group comprises a cDNA ID that also appears in Knock-in or Knock-out tables that causes one or more of the phenotypes described in section above.

30        Reactive Oxygen Genes Identified By Amino Acid Sequence Similarity

Reactive Oxygen genes from other plant species typically encode polypeptides that share amino acid similarity to the sequences encoded by corn and Arabidopsis Reactive Oxygen genes. Groups of Reactive Oxygen genes are identified in the Protein Group table. In this table, any protein group that comprises a peptide ID that corresponds to a cDNA ID member of a Reactive  
5 Oxygen pathway or network is a group of proteins that also exhibits Reactive Oxygen functions/utilities.

Further, promoters of reactive oxygen responsive genes, as described in the Reference tables, for example, are useful to modulate transcription that is induced by reactive oxygen or any of the following phenotypes or biological activities below.

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V.F.1. USE OF REACTIVE OXYGEN RESPONSIVE GENES TO  
MODULATE PHENOTYPES

Reactive oxygen responsive genes and gene products are useful to or modulate one or more of the following phenotypes:

- Pathogen Tolerance and/or Resistance
- Avr/R locus sensitive
- Non-Host sensitive
- HR
- SAR, e.g., where the reactive oxygen responsive gene and products are modulated in conjunction with any of the bacterial, fungal, virus, or other organism listed below
- Bacteria, resistance e.g. to *Erwinia stewartii*, *Pseudomonas syringae*, *Pseudomonas tabaci*, Stuart's wilt, etc.
- Fungal resistance including to downy mildews such as *Sclerophthora macrospora*, *Sclerophthora rayissiae*, *Sclerospora graminicola*, *Peronosclerospora sorghi*, *Peronosclerospora philippinensis*, *Peronosclerospora sacchari*, *Peronosclerospora maydis*; rusts such as *Puccinia sorphi*, *Puccinia polysora*, *Physopella zeae*, etc.; other fungal diseases such as *Cercospora zeae-maydis*, *Colletotrichum graminicola*, *Fusarium moniliforme*, *Exserohilum turcicum*, *Bipolaris maydis*, *Phytophthora parasitica*, *Peronospora tabacina*, *Septoria*, etc.;

- Virus or viroid resistance, e.g. to tobacco or cucumber mosaic virus, ringspot virus, necrosis virus, pelargonium leaf curl virus, red clover mottle virus, tomato bushy stunt virus, and like viruses;
- Insect resistance, such as to aphids e.g. *Myzus persicae*; beetles, beetle larvae; etc.
- nematodes, e.g. *Meloidogyne incognita*; lepidoptera, e.g. *Heliothus* spp. etc.
- Resistance Specifically in Primary or Secondary Leaves
- Stress Tolerance
- Winter Survival
- Cold Tolerance
- Heavy Metal Tolerance, such as Cadmium
- Physical Wounding;
- Increased Organelle Tolerance to Redox Stress, such as in Mitochondria, and chloroplasts
- Cell Death
- Apoptosis, including death of diseased tissue;
- Senescence;
- Fruit Drop;
- Biomass
- Fresh and Dry Weight during any time in plant life, such as maturation;
- Number of Flowers, Seeds, Branches, and/or Leaves;
- Seed Yield, including Number, Size, Weight, and/or Harvest Index
- Fruit Yield, including Number, Size, Weight, and/or Harvest Index
- Plant Development
- Time to Fruit Maturity
- Cell Wall Strengthening and Reinforcement
- Plant Product Quality
  - Paper making quality
- Food additives
- Treatment of Indications modulated by Free Radicals
- Cancer

To regulate any of the phenotype(s) above, activities of one or more of the reactive oxygen

responsive genes or gene products can be modulated and the plants can be tested by screening for the desired trait. Specifically, the gene, mRNA levels, or protein levels can be altered in a plant utilizing the procedures described herein and the phenotypes can be screened for variants as in Winkler et al. (1998) Plant Physiol 118: 743-50 and assayed, for example, in accordance to Alvarez et al., (1998) Cell 92: 773-784; Halhbrock and Scheel, (1989) Ann. Rev. Plant Physiol. Plant Mol. Biol. 40: 347-369; Lamb et al., (1997) Ann. Rev. Plant Mol. Biol. Plant Physio. 48: 251-275; Lapwood et al. (1984) Plant Pathol. 33: 13-20; Levine et al. (1996) Curr. Biol. 6: 427-437; McKersie et al., (2000) Plant Physiol. 122(4): 1427-1437; Olson and Varner (1993) Plant J. 4: 887-892; Pastore et al., (2000), FEBS Lett 470(1): 88-92; Pastori et al., (1997) Plant Physiol. 113: 411-418. Romero-Puertas et al., (1999) Free Radic. Res. 1999 31 Suppl: S25-31; Shirataki et al., Anticancer Res 20(1A): 423-426 (2000); Wu et al., (1995) Plant Cell 7: 1357-1368;

V.F.2. USE OF REACTIVE OXYGEN RESPONSIVE GENES TO  
MODULATE BIOCHEMICAL ACTIVITIES

The activities of one or more of the reactive oxygen responsive genes can be modulated to change biochemical or metabolic activities and/or pathways such as those noted below. Such biological activities are documented and can be measured according to the citations above and included in the Table below:

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
Reinforcement of Cell Walls	Modulation Of The Production Of ExtracTable Proline-Rich Protein	Bradley et al. 1992. Cell 70, 21-30
	Modulation Of Lignification	Mansouri et al. (1999) Physiol Plant 106: 355-362
Stress, Disease, Pathogen Resistance and Wounding	Induction Of Pathogenesis Related Proteins, Phytoalexins And Many Defense Pathways.  Induction Of Detoxifying Enzymes Such As Glutathione S- Transferase And Ascorbate Peroxidase Disease Resistance	Chamnongpol et.al.(1998) Proc. Nat.Acad Sci USA 12;95:5818-23. Davis et al. (1993) Phytochemistry 32: 607-611. Chen et.al. Plant J. (1996) 10:955-966 Gadea et.al.(1999) Mol Gen Genet 262:212-219  Wu et.al.(1995) Plant Cell 7: 1357-68

PROCESS	BIOCHEMICAL OR METABOLIC ACTIVITIES AND/OR PATHWAYS	CITATIONS INCLUDING ASSAYS
	Reactive Oxygen Generation Following Wounding And Changes In Physical Pressure	Orozco-Cardenas and Ryan (1999) Proc.Nat. Acad. Sci. USA 25;96:6553-7. Yahraus et al. (1995) Plant Physiol. 109: 1259-1266
	Modulation Of Genes Involved In Wound Repair And Cell Division	egendre et al. (1993) Plant hysiol. 102: 233-240
	Modulation Of Nitric Oxide Signaling	Delledonne et al. (1998) Nature 394: 585-588
	Salicyclic Acid Accumulation And Signaling	Durner and Klessig (1996) J.Biol.Chem. 271:28492-501
Programmed Cell Death	Induction Of Cell Death Pathway Genes	Levine et al. (1996) Curr. Biol. 6: 427-437. Reynolds et.al.(1998) Biochem.J. 330:115-20

Other biological activities that can be modulated by the reactive oxygen responsive genes and their products are listed in the Reference tables. Assays for detecting such biological activities are described in the Protein Domain table.

5        Reactive oxygen responsive genes are characteristically differentially transcribed in response to fluctuating reactive oxygen levels or concentrations, whether internal or external to an organism or cell. The MA\_diff and/or AFLP\_diff MA\_diff and/or AFLP\_diff and/or AFLP\_diff table reports the changes in transcript levels of various reactive oxygen responsive genes in the aerial parts of a plant at 1 and 6 hours after the plant was sprayed with Silwett L-77  
10        solution enriched with hydrogen peroxide as compared to plants sprayed with Silwett L-77 alone.

The data from this time course reveal a number of types of reactive oxygen responsive genes and gene products, including "early responders," and "delayed responders". Profiles of



individual reactive oxygen responsive genes are shown in the Table below together with examples of which associated biological activities are modulated when the activities of one or more such genes vary in plants.

GENE EXPRESSION LEVELS	FUNCTIONAL CATEGORY OF GENE	PHYSIOLOGICAL CONSequence	EXAMPLES OF BIOCHEMICAL ACTIVITY OF GENE PRODUCTS
Upregulated transcripts (Higher at 1h Than 6h) (Level at 1 h $\cong$ 6h)	Early Responders To Reactive Oxygen	- Perceiving Reactive Oxygen  - Reactive Oxygen Response Transduction Pathways  - Initiating Specific Gene Transcription	-Transcription Factors -Kinases And Phosphatases -Transporters - Glutathione S-Transferase -Heat Shock Proteins -Salicylic Acid Response Pathway Proteins -Jasmonic Acid Pathway Proteins -Dehydrins -Peroxidases -Catalase -Proteases -Pathogen Response Proteins -Ca 2+ Channel Blockers -Phenylalanine Ammonia Lyase
Upregulated transcripts (Lower at 1h Than 6h)	Delayed Reactive Oxygen Responders	Maintenance Of Defence Pathways To Control Active Oxygen  Activation Of Cell	-Transcription Factors - Kinases And Phosphatases - Reactive Oxygen Scavenging Enzymes - Cell Wall And Cell Division/Growth Promoting Pathway Enzymes

		Death Pathways In Specific Cells	<ul style="list-style-type: none"> <li>- Pathogen Response Proteins</li> <li>- Proteins Of Defence Pathways</li> <li>- Proteases, Cellulases, Nucleases And Other Degrading Enzymes.</li> <li>- Membrane Proteins</li> <li>-Mitochondrial And Chloroplast Energy Related Proteins</li> </ul>
<p>Downregulated transcripts</p> <p>Level at 1h <math>\cong</math> 6h</p> <p>Level at 6h &gt; 1h.</p>	<p>Early Responder Repressors Of Reactive Oxygen Response Pathways</p> <p>Genes Of Pathways That Are Minimized In Response To Reactive Oxygen</p> <p>Delayed Responder Repressors Of Reactive Oxygen Response Pathways</p> <p>Genes Of Pathways That</p>	<p>Negative Regulation Of Reactive Oxygen-Inducible Pathways Released</p> <p>Reduction In Activities Of Pathways Not Maintained Under High Reactive Oxygen</p> <p>Negative Regulation Of Reactive Oxygen Inducible Pathways Released</p> <p>Reduction In</p>	<ul style="list-style-type: none"> <li>-Transcription Factors</li> <li>- Kinases And Phosphatases</li> <li>- Chromatin Remodelling Proteins</li> <li>- Metabolic Enzymes In Affected Cells</li> <li>- Membrane Proteins And Cell Wall Proteins</li> <li>-Transcription Factors</li> <li>- Kinases And Phosphatases</li> <li>-Chromatin Remodelling Proteins</li> <li>- Metabolic Enzymes In Affected Cells</li> <li>- Membrane Proteins And Cell Wall Proteins</li> </ul>
<p>Down Regulated Transcripts</p> <p>(Level at 1h &gt; 6 h</p>			

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